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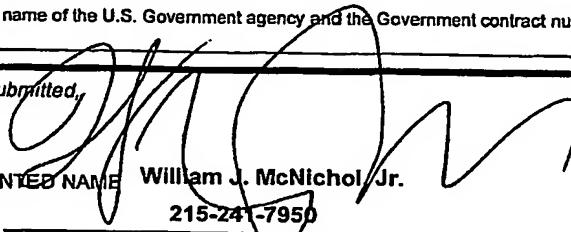
PROVISIONAL APPLICATION FOR PATENT COVER SHEET
This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

PTO

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TITLE OF THE INVENTION (280 characters max)		
A Novel Gene And Uses Therefor		
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ENCLOSED APPLICATION PARTS (check all that apply)		
<input checked="" type="checkbox"/> Specification Number of Pages	104	<input type="checkbox"/> CD(s), Number
<input type="checkbox"/> Drawing(s) Number of Sheets	20	<input checked="" type="checkbox"/> Other (specify) Sequence Listing
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76		
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Respectfully submitted,

SIGNATURE 

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Date 1/13/2003

REGISTRATION NO. 31,179

(if appropriate)

Docket Number: 03-40003-PR

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C.

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January 13, 2003

Commissioner for Patents
BOX Provisional Application
Washington, D.C. 20231

Re: New Provisional Application
For: "A Novel Gene And Uses Therefor"
File Date: January 13, 2003
Our Reference: 03-40003-PR

Dear Sir:

Enclosed for filing in connection with the above-referenced provisional patent application are the following documents:

1. Specification (104 pages);
2. Drawings (20 pages);
3. Sequence Listing (11 pages)
4. Provisional Application Cover Sheet (in duplicate); and
5. A self-addressed stamped postcard, return of which is requested to acknowledge receipt of the enclosed documents.

EXPRESS MAIL CERTIFICATE (37 CFR 1.10)

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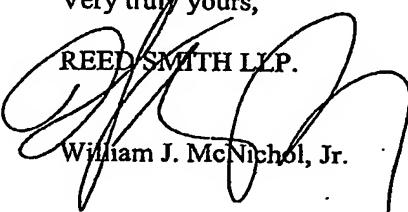
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Very truly yours,

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William J. McNichol, Jr.

Enclosures

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- 1 -

A NOVEL GENE AND USES THEREFOR

BACKGROUND OF THE INVENTION

5 FIELD OF THE INVENTION

The present invention relates generally to a nucleic acid molecule which is expressed in at least red gastrocnemius muscle of *Psammomys obesus* under particular physiological conditions. It is proposed that the nucleic acid molecule is differentially expressed under 10 differing conditions of obesity, anorexia, weight maintenance, diabetes and/or metabolic energy levels. More particularly, the present invention uses microarray technology to identify genes which are expressed under particular physiological conditions. It is proposed that the subject nucleic acid molecule and/or its expression product be used in therapeutic and diagnostic protocols for conditions such as obesity, anorexia, weight maintenance, diabetes 15 and/or energy imbalance. The subject nucleic acid molecule and its expression product or derivatives, homologs, analogs and mimetics thereof are proposed to be useful, therefore, as therapeutic and diagnostic agents for obesity, anorexia, weight maintenance, diabetes and/or energy imbalance or as targets for the design and/or identification of modulators of their activity and/or function.

20

DESCRIPTION OF THE PRIOR ART

Bibliographic details of references provided in the subject specification are listed at the end of the specification.

25

Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

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The increasing sophistication of recombinant DNA technology is greatly facilitating research and development in the medical, veterinary and allied human and animal health fields. This is particularly the case in the investigation of the genetic bases involved in the etiology of certain disease conditions. One particularly significant condition from the stand point of morbidity
5 and mortality is obesity and its association with type 2 diabetes (formerly non-insulin-dependent diabetes mellitus or NIDDM) and cardiovascular disease.

Obesity is defined as a pathological excess of body fat and is the result of an imbalance between energy intake and energy expenditure for a sustained period of time. Obesity is the
10 most common metabolic disease found in affluent societies. The prevalence of obesity in these nations is alarmingly high, ranging from 10% to upwards of 50% in some sub-populations (Bouchard, *The genetics of Obesity*, Boca Raton: CRC Press, 1994). Of particular concern is the fact that the prevalence of obesity appears to be rising consistently in affluent societies and is now increasing rapidly in less prosperous nations as they become more affluent and/or
15 adopt cultural practices similar to those in more affluent countries (Zimmet, *Diabetes Care* 15: 232-252, 1992). The escalating rates of obesity globally have resulted in the World Health Organisation declaring an obesity epidemic worldwide (World Trade Organisation. *Obesity. Preventing and managing the global epidemic. Report of a WHO Consultation on Obesity*. Geneva: World Health Organisation, 1998).

20

In Australia, the recent AusDiab study estimated that 7.5 million Australians (60%) aged 25 years and over were overweight or obese. Of these, 2.6 million (21%) were obese ($BMI > 30$) (Dunstan *et al.*, *Diabetes Res. Clin. Pract.* 57: 119-129, 2002). Similarly, the prevalence of obesity in the U.S. increased substantially between 1991 and 1998, increasing from 12% to
25 18% in Americans during this period (Mokdad *et al.*, *JAMA* 282(16): 1519-1522, 1999).

The high and increasing prevalence of obesity has serious health implications for both individuals and society as a whole. Obesity is a complex and heterogeneous disorder and has been identified as a key risk indicator of preventable morbidity and mortality as obesity

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increases the risk of a number of other metabolic conditions including type 2 diabetes mellitus and cardiovascular disease (Must *et al.*, *JAMA* 282(16): 1523-1529, 1999; Kopelman, *Nature* 404: 635-643, 2000). Alongside obesity the prevalence of diabetes continues to increase rapidly. The AusDiab survey estimated that close to 1 million Australians aged 25 years and 5 over have type 2 diabetes (Dunstan *et al.*, 2002). This represents approximately 7.5% of the population. In the U.S., the number of adults with diabetes increased by 49% between 1991 and 2000 (Marx, *Science* 686-689, 2002). It has been estimated that about 17 million people in the U.S. have type 2 diabetes and an equal number are thought to be pre-diabetic (Marx, 2002). In Australia, the annual costs of obesity associated with diabetes and other disease 10 conditions has been conservatively estimated to be AUS\$810million for 1992-93 (National Health and Medical Research Council, *Acting on Australia's weight: A strategy for the prevention of overweight and obesity*. Canberra: National Health and Medical Research Council, 1996). The direct costs of diabetes and its complications in Australia in 1993-94 were estimated at \$681 million, or 2.2% of total health system costs in that year (Australian Institute 15 of Health and Welfare (AIWH), *Australia's Health*, 2002, Canberra: AIWH).

A genetic basis for the etiology of obesity is indicated *inter alia* from studies in twins, adoption studies and population-based analyses which suggest that genetic effects account for 25-80% of the variation in body weight in the general population (Bouchard, 1994, *supra*; 20 Kopelman *et al.*, *Int. J. Obesity* 18: 188-191, 1994; Ravussin, *Metabolism* 44(3): 12-14, 1995). It is considered that genes determine the possible range of body weight in an individual and then the environment influences the point within this range where the individual is located at any given time (Bouchard, 1994). However, despite numerous studies into genes thought to be involved in the pathogenesis of obesity, there have been surprisingly few significant 25 findings in this area. In addition, genome-wide scans in various population groups have not produced definitive evidence of the chromosomal regions having a major effect on obesity.

A number of tissues have been implicated in the pathophysiology of obesity and type 2 diabetes, and of particular interest is the muscle. Skeletal muscle is the principle site of

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insulin-stimulated glucose disposal, accounting for approximately 75% of total glucose uptake. Skeletal muscle is also the major site of peripheral insulin resistance. Skeletal muscle also oxidizes free fatty acids for fuel, to meet its energy requirements. In healthy individuals, the muscle has the capacity to utilize both carbohydrate and lipids for energy and to fluctuate
5 between these fuels in response to a range of signals including insulin concentrations. This metabolic flexibility is central to the role the muscle plays in whole body fuel metabolism and with diseases such as obesity and type 2 diabetes, this flexibility may be lost.

In accordance with the present invention, genetic sequences were sought which are expressed
10 in at least red gastrocnemius muscle of *Psammomys obesus* (Israeli Sand Rat) under particular physiological conditions. Novel genes are identified which have human and/or murine equivalents or homologs. In accordance with the present invention, genes are isolated which are proposed to be associated with one or more biological functions associated with disease conditions such as but not limited to obesity, anorexia, diabetes or energy balance.

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SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a

5 stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID

NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1 (SEQ ID

10 NO:1), <400>2 (SEQ ID NO:2), etc. A summary of the sequence identifiers is provided in Table 3. A sequence listing is provided after the claims. A summary of genes identified in accordance with the present invention is provided in Table 1. Gene abbreviations are provided in Table 2.

15 Analysis of genetic material from red gastrocnemius muscle tissue were used to identify candidate genetic sequences associated with a healthy state or with physiological conditions such as obesity, anorexia, weight maintenance, diabetes and/or metabolic energy levels. An animal model was employed comprising the Israeli Sand Rat (*Psammomys obesus*). Three groups of animals were used designated Groups A, B and C based on metabolic phenotype as
20 follows:-

Group A: lean animals (normoglycemic; normoinsulinemic);

Group B: obese, non-diabetic animals (normoglycemic; hyperinsulinemic); and

Group C: obese, diabetic animals (hyperglycemic; hyperinsulinemic).

25

Microarray analysis was used to identify genetic sequences in fed and fasted mammals or in exercise trained and control mammals. The Israeli Sand Rat (*Psammomys obesus*) was found to be particularly useful for this analysis.

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cDNA microarray technology provides a powerful technical means to generate a gene expression database of both known genes and unknown transcripts. Using cDNA microarrays, comparative estimates can be obtained of the level of gene expression of large numbers of genes (up to 20,000 per microarray) in each sample. cDNA microarrays generally involve a

5 large number of DNA "spots" in an orderly array chemically coupled to the surface of a solid substrate, usually but not exclusively an optically flat glass microscope slide. Fluorescently labeled cDNAs are generated from experimental and reference RNA samples and then competitively hybridized to the gene chip. The experimental and reference cDNAs are labeled with a different fluorescent dye and the intensity of each fluor at each DNA spot gives an

10 indication of the level of that particular RNA species in the experimental sample relative to the reference RNA. The ratio of fluorescence can be taken as a measure of the expression level of the gene corresponding to that spot in the experimental sample.

In a preferred embodiment, nine expressed sequences were identified designated herein *AGT*-
15 701 [SEQ ID NO:1], *AGT*-702 [SEQ ID NO:2], *AGT*-704 [SEQ ID NO:3], *AGT*-705 [SEQ ID NO:4], *AGT*-706 [SEQ ID NO:5], *AGT*-707 [SEQ ID NO:6], *AGT*-708 [SEQ ID NO:7], *AGT*-
709 [SEQ ID NO:8] and *AGT*-710 [SEQ ID NO:9].

A summary of the *AGT* genes is provided in Table 1.
20

The present invention contemplates the use of these sequences or their expression products in the manufacture of medicaments and diagnostic agents for a range of conditions including obesity, anorexia, weight maintenance, diabetes and/or energy imbalance.

25 The present invention provides, therefore, a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an expression product or a derivative, homolog, analog or mimetic thereof wherein said nucleic acid molecule or its homolog is differentially expressed in red gastrocnemius of *P. obesus* under fed or fasted or in exercise trained and control conditions.

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The present invention further provides a nucleic acid molecule comprising a nucleotide sequence encoding or complementary to a sequence encoding an expression product or a derivative, homolog, analog or mimetic thereof wherein the nucleotide sequence is as

5 substantially set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5 or SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9 or a nucleotide sequence having at least about 30% similarity to all or part of SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5 or SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9 and/or is capable of hybridizing to one or more of

10 SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5 or SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9 or their complementary forms under low stringency conditions at 42°C and wherein the nucleic acid molecule is differentially expressed in red gastrocnemius tissue of *P. obesus* under fed or fasted or in exercise trained and control conditions.

15

The present invention also provides an isolated expression product or a derivative, homolog, analog or mimetic thereof which expression product is encoded by a nucleotide sequence which is differentially expressed in red gastrocnemius tissue of *P. obesus* under fed or fasted or in exercise trained and control conditions.

20

More particularly, the present invention is directed to an isolated expression product or a derivative, homolog, analog or mimetic thereof wherein the expression product is encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5 or SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9 or a nucleotide sequence having at least 30% similarity to all or part of SEQ

25 ID NO:1, SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5 or SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9 and/or is capable of hybridizing to SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5 or SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9 or their complementary forms under low

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stringency conditions at 42°C.

The preferred genetic sequence of the present invention are referred to herein as *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710*. The expression products encoded by *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* are referred to herein as *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710*, respectively. The expression product may be an RNA (e.g. mRNA) or a protein. Where the expression product is an RNA, the present invention extends to RNA-related molecules associated thereto such as
10 RNAi or intron or exon sequences therefrom.

Even yet another aspect of the present invention relates to a composition comprising *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and/or *AGT-710* or its derivatives, homologs, analogs or mimetics or agonists or antagonists of *AGT-701*,
15 *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and/or *AGT-710* together with one or more pharmaceutically acceptable carriers and/or diluents.

The present invention is particularly directed to human homologs of the genes identified in *P. obesus* and their use in therapy and diagnosis.
20

Another aspect of the present invention contemplates, therefore, a method for treating a subject comprising administering to said subject a treatment effective amount of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and/or *AGT-710* or a derivative, homolog, analog or mimetic thereof or a genetic sequence encoding same or
25 an agonist or antagonist of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and/or *AGT-710* activity or *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and/or *AGT-710* gene expression for a time and under conditions sufficient to effect treatment.

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In accordance with this and other aspects of the present invention, treatments contemplated herein include but are not limited to obesity, anorexia, weight maintenance, energy imbalance and diabetes. Treatment may be by the administration of a pharmaceutical composition or genetic sequences *via* gene therapy. Treatment is contemplated for human subjects as well as
5 animals such as animals important to livestock industry.

A further aspect of the present invention is directed to a diagnostic agent for use in monitoring or diagnosing conditions such as but not limited to obesity, anorexia, weight maintenance, energy imbalance and/or diabetes, said diagnostic agent selected from an antibody to AGT-
10 701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-710 or its derivatives, homologs, analogs or mimetics and a genetic sequence comprising or capable of annealing to a nucleotide strand associated with *AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 or AGT-710* useful *inter alia* in PCR, hybridization, RFLP analysis or AFLP analysis.

- 10 -

TABLE 1
Summary of AGT Genes

GENE	SEQ ID NO.	HOMOLOG	DESCRIPTION
AGT-701	1	human, mouse and rat NDRG2	lower expression in Group C, fed animals and higher expression in Group B fasted animals; expression negatively correlated with body fat, body weight and blood glucose in fed animals; increases with exercise training
AGT-702	2	human, mouse and rat PRSS11	elevated expression after training; negative correlation with body weight and blood glucose; positive correlation with energy expenditure
AGT-704	3	human PAI-RBP1	decreased expression in Group C, fed animals; negative correlation with blood glucose in fed animals; increases with exercise training
AGT-705	4	murine BC030414	increased expression in Group C animals and in Group B fasted animals; expression negatively correlated with blood glucose; increases with exercise training
AGT-706	5	human FL520069 murine Ahi-1	elevated expression in Group B fasted and Group C fasted animals; expression negatively correlated with blood glucose in fed animals and positively correlated in insulin in fasted animals; increases with exercise training
AGT-707	6	human ASNA1	elevated expression in Group A animals; expression negatively correlated with body weight in fed animals; increases with exercise training
AGT-708	7	human PKIA	elevated expression in Group B and Group C fasted animals; expression positively correlated with blood glucose

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GENE	SEQ ID NO.	HOMOLOG	DESCRIPTION
AGT-709	8	human KIAA0633, Mus musculus similar to KIAA0633	lower expression in Group C, fed animals; expression negatively correlated with body weight and blood glucose
AGT-710	9	human, mouse and rat SCP2	lower expression in Group C, fed animals; expression negatively correlated with body weight and blood glucose in fed animals; increases with exercise training

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TABLE 2
Gene Abbreviations

ABBREVIATION	DEFINITION
NDRG2	N-myc downstream-regulated gene 2
PRSS11	protease, serine 11
PAI-RBP1	PAI-1 mRNA binding protein
ASNA1	human homolog of bacterial arsA arsenite transporter ATP binding
PKIA	protein kinase inhibitor alpha
SCP2	sterol carrier protein 2

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A summary of sequence identifiers used throughout the subject specification is provided in Table 3.

TABLE 3
Summary of Sequence Identifiers

SEQUENCE ID. NO.	DESCRIPTION
1	Nucleotide sequence of <i>AGT-701</i>
2	Nucleotide sequence of <i>AGT-702</i>
3	Nucleotide sequence of <i>AGT-704</i>
4	Nucleotide sequence of <i>AGT-705</i>
5	Nucleotide sequence of <i>AGT-706</i>
6	Nucleotide sequence of <i>AGT-707</i>
7	Nucleotide sequence of <i>AGT-708</i>
8	Nucleotide sequence of <i>AGT-709</i>
9	Nucleotide sequence of <i>AGT-710</i>
10	primer SP6
11	primer T7
12	peptide
13	peptide

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphical representation showing *AGT-701* gene expression in *P. obesus* red gastrocnemius muscle after exercise. * $p < 0.001$.

5

Figure 2 is a graphical representation of *AGT-701* gene expression *versus* blood glucose in all animals.

10 **Figure 3** is a graphical representation of *AGT-701* gene expression *versus* change in blood glucose in all animals.

Figure 4 is a graphical representation of *AGT-701* gene expression *versus* energy expenditure in all animals.

15 **Figure 5** is a graphical representation showing *AGT-701* gene expression in fed animals. * $p = 0.011$.

Figure 6 is a graphical representation showing expression of *AGT-701* in fasted animals. * $p = 0.04$.

20

Figure 7 is a graphical representation showing expression of *AGT-701* *versus* percent body fat in fed animals.

25 **Figure 8** is a graphical representation showing expression of *AGT-701* *versus* body weight in fed animals.

Figure 9 is a graphical representation showing expression of *AGT-701* *versus* blood glucose in fed animals.

- 15 -

Figure 10 is a graphical representation of *AGT-702* gene expression in *P. obesus* red gastrocnemius muscle after exercise. * $p < 0.001$.

5 **Figure 11** is a graphical representation of *AGT-702* gene expression *versus* body weight in exercise trained animals.

Figure 12 is a graphical representation of *AGT-701* gene expression *versus* energy expenditure in all animals.

10 **Figure 13** is a graphical representation of *AGT-702* gene expression *versus* blood glucose in all animals.

Figure 14 is a graphical representation of *AGT-702* gene expression *versus* change in blood glucose in all animals.

15 **Figure 15** is a graphical representation of *AGT-704* gene expression in *P. obesus* red gastrocnemius muscle after exercise. * $p < 0.001$.

20 **Figure 16** is a graphical representation of *AGT-704* gene expression *versus* blood glucose in all animals.

Figure 17 is a graphical representation of *AGT-704* gene expression *versus* energy expenditure in all animals.

25 **Figure 18** is a graphical representation of *AGT-704* gene expression in fed animals.

Figure 19 is a graphical representation of *AGT-704* gene expression *versus* blood glucose in fed animals.

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Figure 20 is a graphical representation of *AGT-705* gene expression in *P. obesus* red gastrocnemius muscle after exercise.

Figure 21 is a graphical representation of *AGT-705* gene expression in fed animals.

5

Figure 22 is a graphical representation of *AGT-705* gene expression in fasted animals. *p = 0.035, #p = 0.007.

10 **Figure 23** is a graphical representation of *AGT-705* gene expression *versus* blood glucose in fasted animals.

Figure 24 is a graphical representation of *AGT-706* gene expression in *P. obesus* red gastrocnemius muscle after exercise. *p = 0.001.

15 **Figure 25** is a graphical representation of *AGT-706* gene expression *versus* blood glucose in all animals.

Figure 26 is a graphical representation of *AGT-706* gene expression *versus* insulin in all animals.

20

Figure 27 is a graphical representation of *AGT-704* gene expression *versus* energy expenditure in all animals.

25 **Figure 28** is a graphical representation of *AGT-706* gene expression in fasted animals. *p < 0.001, #p < 0.001.

Figure 29 is a graphical representation of *AGT-706* gene expression *versus* blood glucose in fed animals.

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Figure 30 is a graphical representation of *AGT-706* gene expression *versus* insulin in fasted animals.

5 **Figure 31** is a graphical representation of *AGT-707* gene expression in *P. obesus* red gastrocnemius muscle after exercise. **p* = 0.037.

Figure 32 is a graphical representation of *AGT-707* gene expression in fed animals.

10 **Figure 33** is a graphical representation of *AGT-707* gene expression *versus* body weight in fed animals.

Figure 34 is a graphical representation of *AGT-708* gene expression *versus* activity in all animals.

15 **Figure 35** is a graphical representation of *AGT-708* gene expression *versus* energy expenditure in all animals.

Figure 36 is a graphical representation of *AGT-708* gene expression *versus* change in carbohydrate oxidation in all animals.

20

Figure 37 is a graphical representation of *AGT-708* gene expression *versus* change in fat oxidation in all animals.

25 **Figure 38** is a graphical representation of *AGT-708* gene expression *versus* food intake in exercise trained animals.

Figure 39 is a graphical representation of *AGT-708* gene expression *versus* change in fat oxidation in exercise trained animals.

- 18 -

Figure 40 is a graphical representation of *AGT-708* gene expression *versus* change in carbohydrate oxidation in exercise trained animals.

5 **Figure 41** is a graphical representation of *AGT-708* gene expression in fasted animals. *p = 0.01.

Figure 42 is a graphical representation of *AGT-708* gene expression *versus* blood glucose in fasted animals.

10 **Figure 43** is a graphical representation of *AGT-709* gene expression *versus* body weight in all animals.

15 **Figure 44** is a graphical representation of *AGT-709* gene expression *versus* energy expenditure in all animals.

Figure 45 is a graphical representation of *AGT-709* gene expression *versus* body weight in exercise trained animals.

20 **Figure 46** is a graphical representation of *AGT-709* gene expression *versus* change in blood glucose in exercise trained animals.

Figure 47 is a graphical representation of *AGT-709* gene expression *versus* change in body weight in exercise trained animals.

25 **Figure 48** is a graphical representation of *AGT-709* gene expression in fed animals.

Figure 49 is a graphical representation of *AGT-709* gene expression *versus* body weight in fed animals.

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Figure 50 is a graphical representation of *AGT-709* gene expression *versus* blood glucose in fed animals.

5 **Figure 51** is a graphical representation of *AGT-710* gene expression in *P. obesus* red gastrocnemius muscle after exercise. **p* = .0016.

Figure 52 is a graphical representation of *AGT-710* gene expression *versus* energy expenditure in all animals.

10 **Figure 53** is a graphical representation of *AGT-710* gene expression *versus* blood glucose in all animals.

Figure 54 is a graphical representation of *AGT-710* gene expression *versus* activity in all animals.

15 **Figure 55** is a graphical representation of *AGT-710* gene expression *versus* fat oxidation in exercise trained animals.

20 **Figure 56** is a graphical representation of *AGT-710* gene expression *versus* change in fat oxidation in exercise trained animals.

Figure 57 is a graphical representation of *AGT-710* gene expression in fed animals. **p* = 0.021.

25 **Figure 58** is a graphical representation of *AGT-710* gene expression *versus* body weight in fed animals.

Figure 59 is a graphical representation of *AGT-710* gene expression *versus* blood glucose in fed animals.

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DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated in part on the identification of novel genes associated *inter alia* with regulation of obesity, anorexia, weight maintenance, diabetes and/or metabolic energy levels. The genes are identified following differential screening of mRNA from red gastrocnemius tissue in fed and fasted animals or in exercise trained and control *P. obesus* animals. The selection of gastrocnemius tissue is not intended to imply that differential expression does not occur in other tissue. The present invention further extends to homologs in other mammals and in particular humans as well as in other animals or organisms.

10

Accordingly, one aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an expression product or a derivative, homolog, analog or mimetic thereof wherein said nucleic acid molecule is differentially expressed in red gastrocnemius muscle tissue of *P. obesus* under fed and fasted or in exercise trained and control conditions or a homolog of said nucleic acid molecule.

The term "differentially expressed" is used in its most general sense and includes elevated levels of an expression product such as mRNA or protein or a secondary product such as cDNA in one tissue compared to another tissue or in the same tissue but under different conditions. Examples of different conditions includes differential expression in tissue from fed and fasted animals or in exercise trained and control animals. Differential expression is conveniently determined by a range of techniques including polymerase chain reaction (PCR) such as real-time PCR. Other techniques include suppression subtractive hybridization (SSH) and amplified fragment length polymorphism (AFLP) analysis. Microarray analysis of cDNA is particularly preferred.

A homolog refers to a genetic sequence in another animal or organism which has at least about 20% identity to the reference sequence. A preferred homolog is a human homolog.

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Conveniently, an animal model may be employed to study the differences in gene expression in animal tissues under different conditions. In particular, the present invention is exemplified using the *P. obesus* (the Israeli Sand Rat) animal model of dietary-induced obesity and type 2 diabetes. In their natural desert habitat, an active lifestyle and saltbush diet ensure that they remain lean and normoglycemic (Shafir and Gutman, *J. Basic Clin. Physiol. Pharm.* 4: 83-99, 1993). However, in a laboratory setting on a diet of *ad libitum* chow (on which many other animal species remain healthy), a range of pathophysiological responses are seen (Barnett *et al.*, *Diabetologia* 37: 671-676, 1994a; Barnett *et al.*, *Int. J. Obesity* 18: 789-794, 1994b; 5 Barnett *et al.*, *Diabete Nutr. Metab.* 8: 42-47, 1995). By the age of 16 weeks, more than half of the animals become obese and approximately one third develop type 2 diabetes. Only hyperphagic animals go on to develop hyperglycemia, highlighting the importance of excessive energy intake in the pathophysiology of obesity and type 2 diabetes in *P. obesus* (Collier *et al.*, *Ann. New York Acad. Sci.* 827: 50-63, 1997a; Walder *et al.*, *Obesity Res.* 5: 10 193-200, 1997a). Other phenotypes found include hyperinsulinemia, dyslipidemia and impaired glucose tolerance (Collier *et al.*, 1997; Collier *et al.*, *Exp. Clin. Endocrinol. Diabetes* 105: 36-37, 1997b). *P. obesus* exhibit a range of bodyweight and blood glucose and insulin levels which form a continuous curve that closely resembles the patterns found in human populations, including the inverted U-shaped relationship between blood glucose and insulin 15 levels known as "Starling's curve of the pancreas" (Barnett *et al.*, 1994a). It is the heterogeneity of the phenotypic response of *P. obesus* which makes it an ideal model to study the etiology and pathophysiology of obesity and type 2 diabetes.

20

The animals are conveniently classified into three groups designated Groups A, B and C:

25

- Group A: animals are lean;
- Group B: animals are obese and non-diabetic; and
- Group C: animals are obese and diabetic.

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In accordance with the present invention, a number of differentially expressed genetic sequences were identified in red gastrocnemius tissue in *P. obesus* under different feeding regimes (i.e. fed and fasted) or under exercise trained and control conditions. These genetic sequences have human and other animal homologs and, hence, the identification of these

5 genetic sequences permits identification of genes involved in obesity, diabetes and energy balance.

Another aspect of the present invention provides a nucleic acid molecule comprising a nucleotide sequence encoding or complementary to a sequence encoding an expression product or a derivative, homolog, analog or mimetic thereof wherein said nucleotide sequence is as substantially set forth in SEQ ID NO:1 (AGT-701) or SEQ ID NO:2 (AGT-702) or SEQ 10 ID NO:3 (AGT-704) or SEQ ID NO:4 (AGT-705) or SEQ ID NO:5 (AGT-706) or SEQ ID NO:6 (AGT-707) or SEQ ID NO:7 (AGT-708) or SEQ ID NO:8 (AGT-709) or SEQ ID NO:9 (AGT-710) or a nucleotide sequence having at least about 30% similarity to all or part of SEQ 15 ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5 or SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9 and/or is capable of hybridizing to one or more of SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5 or SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9 or their complementary forms under low stringency conditions at 42°C and wherein said nucleic acid 20 molecule is differentially expressed in red gastrocnemius muscle tissue under fed or fasted or in exercise trained and control conditions.

Higher similarities are also contemplated by the present invention such as greater than about 40% or 50% or 60% or 70% or 80% or 90% or 95% or 96% or 97% or 98% or 99% or above.

25

An expression product includes an RNA molecule such as an mRNA transcript as well as a protein. Some genes are non-protein encoding genes and produce mRNA or other RNA molecules and are involved in regulation by RNA:DNA, RNA:RNA or RNA:protein interaction. The RNA (e.g. mRNA) may act directly or via the induction of other molecules

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such as RNAi or via products mediated from splicing events (e.g. exons or introns). Other genes encode mRNA transcripts which are then translated into proteins. A protein includes a polypeptide. The differentially expressed nucleic acid molecules, therefore, may encode mRNAs only or, in addition, proteins. Both mRNAs and proteins are forms of "expression products".

Reference herein to similarity is generally at a level of comparison of at least 15 consecutive or substantially consecutive nucleotides. It is particularly convenient, however, to determine similarity by comparing a total or complete sequence, after optimal alignment.

10

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which may encode different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In 15 a particularly preferred embodiment, nucleotide sequence comparisons are made at the level of identity rather than similarity.

Terms used to describe sequence relationships between two or more polynucleotides include "reference sequence", "comparison window", "sequence similarity", "sequence identity", 20 "percentage of sequence similarity", "percentage of sequence identity", "substantially similar" and "substantial identity". A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 or above, such as 30 monomer units in length. Because two polynucleotides may each comprise (1) a sequence (i.e. only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent 25 between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of typically 12 contiguous residues that is compared to a reference sequence. The comparison window may comprise additions or

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deletions (i.e. gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin

5 Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (i.e. resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.* (*Nucl. Acids Res.* 25: 3389, 1997). A detailed discussion of

10 sequence analysis can be found in Unit 19.3 of Ausubel *et al.* ("Current Protocols in Molecular Biology" John Wiley & Sons Inc, Chapter 15, 1994-1998). A range of other algorithms may be used to compare the nucleotide and amino acid sequences such as but not limited to PILEUP, CLUSTALW, SEQUENCHER or VectorNTI.

15 The terms "sequence similarity" and "sequence identity" as used herein refers to the extent that sequences are identical or functionally or structurally similar on a nucleotide-by-nucleotide basis over a window of comparison. Thus, a "percentage of sequence identity", for example, is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g.

20 A, T, C, G, I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, "sequence identity" will be understood to mean the "match percentage" calculated by the DNASIS computer program (Version 2.5 for

25 windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the software. Similar comments apply in relation to sequence similarity.

Reference herein to a low stringency includes and encompasses from at least about 0 to at least about 15% v/v formamide and from at least about 1 M to at least about 2 M salt for hybridization, and at least about 1 M to at least about 2 M salt for washing conditions. Generally, low stringency is at from about 25-30°C to about 42°C. The temperature may be
5 altered and higher temperatures used to replace formamide and/or to give alternative stringency conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridization, and at least about 0.5 M to at least about 0.9 M salt for washing conditions, or
10 high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01 M to at least about 0.15 M salt for hybridization, and at least about 0.01 M to at least about 0.15 M salt for washing conditions. In general, washing is carried out $T_m = 69.3 + 0.41 (G+C)\%$ (Marmur and Doty, *J. Mol. Biol.* 5: 109, 1962). However, the T_m of a duplex DNA decreases by 1°C with every increase of 1%
15 in the number of mismatch base pairs (Bonner and Laskey, *Eur. J. Biochem.* 46: 83, 1974). Formamide is optional in these hybridization conditions. Accordingly, particularly preferred levels of stringency are defined as follows: low stringency is 6 x SSC buffer, 0.1% w/v SDS at 25-42°C; a moderate stringency is 2 x SSC buffer, 0.1% w/v SDS at a temperature in the range 20°C to 65°C; high stringency is 0.1 x SSC buffer, 0.1% w/v SDS at a temperature of at least
20 65°C.

The nucleotide sequence or amino acid sequence of the present invention may correspond to exactly the same sequence of the naturally occurring gene (or corresponding cDNA) or protein or other expression product or may carry one or more nucleotide or amino acid substitutions, additions and/or deletions. The nucleotide sequences set forth in SEQ ID NO:1 (*AGT-701*), SEQ ID NO:2 (*AGT-702*) and SEQ ID NO:3 (*AGT-704*) or SEQ ID NO:4 (*AGT-705*) or SEQ ID NO:5 (*AGT-706*) or SEQ ID NO:6 (*AGT-707*) or SEQ ID NO:7 (*AGT-708*) or SEQ ID NO:8 (*AGT-709*) or SEQ ID NO:9 (*AGT-710*) correspond to novel genes referred to in parenthesis. The corresponding expression products are *AGT-701*, *AGT-702*, *AGT-704*,

AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710. Reference herein to *AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710* includes, where appropriate, reference to the genomic gene or cDNA as well as any naturally occurring or induced derivatives. Apart from the substitutions, deletions and/or additions to the nucleotide sequence, the present invention further encompasses mutants, fragments, parts and portions of the nucleotide sequence corresponding to *AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710*.

Another aspect of the present invention provides a nucleic acid molecule or derivative, homolog or analog thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a sequence encoding an expression product wherein said nucleotide sequence is substantially as set forth in SEQ ID NO:1 (*AGT-701*) or a derivative, homolog or mimetic thereof or having at least about 30% similarity to all or part of SEQ ID NO:1 or a nucleotide sequence capable of hybridizing to SEQ ID NO:1 or its complementary form under low stringency conditions.

Yet another aspect of the present invention provides a nucleic acid molecule or derivative, homolog or analog thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a sequence encoding an expression product wherein said nucleotide sequence is substantially as set forth in SEQ ID NO:2 (*AGT-702*) or a derivative, homolog or mimetic thereof or having at least about 30% similarity to all or part of SEQ ID NO:2 or a nucleotide sequence capable of hybridizing to SEQ ID NO:2 or its complementary form under low stringency conditions.

Still yet another aspect of the present invention provides a nucleic acid molecule or derivative, homolog or analog thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a sequence encoding an expression product wherein said nucleotide sequence is substantially as set forth in SEQ ID NO:3 (*AGT-704*) or a derivative, homolog or mimetic thereof or having at least about 30% similarity to all or part of SEQ ID

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NO:3 or a nucleotide sequence capable of hybridizing to SEQ ID NO:3 or their complementary forms under low stringency conditions.

Even yet another aspect of the present invention provides a nucleic acid molecule or derivative, homolog or analog thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a sequence encoding an expression product wherein said nucleotide sequence is substantially as set forth in SEQ ID NO:4 (*AGT-705*) or a derivative, homolog or mimetic thereof or having at least about 30% similarity to all or part of SEQ ID NO:4 or a nucleotide sequence capable of hybridizing to SEQ ID NO:4 or its complementary form under low stringency conditions.

Even still another aspect of the present invention provides a nucleic acid molecule or derivative, homolog or analog thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a sequence encoding an expression product wherein said nucleotide sequence is substantially as set forth in SEQ ID NO:5 (*AGT-706*) or a derivative, homolog or mimetic thereof or having at least about 30% similarity to all or part of SEQ ID NO:5 or a nucleotide sequence capable of hybridizing to SEQ ID NO:5 or its complementary form under low stringency conditions.

Another aspect of the present invention provides a nucleic acid molecule or derivative, homolog or analog thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a sequence encoding an expression product wherein said nucleotide sequence is substantially as set forth in SEQ ID NO:6 (*AGT-707*) or a derivative, homolog or mimetic thereof or having at least about 30% similarity to all or part of SEQ ID NO:6 or a nucleotide sequence capable of hybridizing to SEQ ID NO:6 or its complementary form under low stringency conditions.

A further aspect of the present invention provides a nucleic acid molecule or derivative, homolog or analog thereof comprising a nucleotide sequence encoding, or a nucleotide

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sequence complementary to a sequence encoding an expression product wherein said nucleotide sequence is substantially as set forth in SEQ ID NO:7 (*AGT-708*) or a derivative, homolog or mimetic thereof or having at least about 30% similarity to all or part of SEQ ID NO:7 or a nucleotide sequence capable of hybridizing to SEQ ID NO:7 or its complementary form under low stringency conditions.

Yet another aspect of the present invention provides a nucleic acid molecule or derivative, homolog or analog thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a sequence encoding an expression product wherein said nucleotide sequence is substantially as set forth in SEQ ID NO:8 (*AGT-709*) or a derivative, homolog or mimetic thereof or having at least about 30% similarity to all or part of SEQ ID NO:8 or a nucleotide sequence capable of hybridizing to SEQ ID NO:8 or its complementary form under low stringency conditions.

Still another aspect of the present invention provides a nucleic acid molecule or derivative, homolog or analog thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a sequence encoding an expression product wherein said nucleotide sequence is substantially as set forth in SEQ ID NO:9 (*AGT-710*) or a derivative, homolog or mimetic thereof or having at least about 30% similarity to all or part of SEQ ID NO:9 or a nucleotide sequence capable of hybridizing to SEQ ID NO:9 or its complementary form under low stringency conditions.

The expression pattern of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* has been determined, *inter alia*, to indicate an involvement in the regulation of one or more of obesity, anorexia, weight maintenance, diabetes and/or energy metabolism. In addition to the differential expression of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* in red gastrocnemius muscle of fed *versus* fasted or exercise trained *versus* control animals, these genes may also be expressed in other tissues including but in no way limited to brain, muscle, adipose tissue,

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pancreas and gastrointestinal tract. The nucleic acid molecule corresponding to each of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* or *AGT-710* is preferably a DNA such as a cDNA sequence or a genomic DNA. A genomic sequence may also comprise exons and introns. A genomic sequence may also include a promoter region or
5 other regulatory regions.

A homolog is considered to be a gene from another animal species which has the same or greater than 30% similarity to one of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* and/or which has a similar function. The above-mentioned genes are exemplified herein from *P. obesus* red gastrocnemius muscle. The present invention extends, however, to the homologous gene, as determined by nucleotide sequence and/or function, from humans, primates, livestock animals (e.g. cows, sheep, pigs, horses, donkeys), laboratory test animals (e.g. mice, guinea pigs, hamsters, rabbits), companion animals (e.g. cats, dogs) and captured wild animals (e.g. rodents, foxes, deer, 10 kangaroos). Homologs may also be present in microorganisms and *C. elegans*.

The nucleic acids of the present invention and in particular *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* and their derivatives and homologs may be in isolated or purified form and/or may be ligated to a vector such as an expression vector. Expression may be in a eukaryotic cell line (e.g. mammalian, insect or yeast cells) or in prokaryote cells (e.g. *E. coli*) or in both. By "isolated" is meant a nucleic acid molecule having undergone at least one purification step and this is conveniently defined, for example, by a composition comprising at least about 10% subject nucleic acid molecule, preferably at least about 20%, more preferably at least about 30%, still more preferably at least 20, about 40-50%, even still more preferably at least about 60-70%, yet even still more preferably 80-90% or greater of subject nucleic acid molecule relative to other components as determined by molecular weight, encoding activity, nucleotide sequence, base composition or other convenient means. The nucleic acid molecule of the present invention may also be considered, in a preferred embodiment, to be biologically pure. The nucleic acid molecule may be ligated
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to an expression vector capable of expression in a prokaryotic cell (e.g. *E. coli*) or a eukaryotic cell (e.g. yeast cells, fungal cells, insect cells, mammalian cells or plant cells). The nucleic acid molecule may be ligated or fused or otherwise associated with a nucleic acid molecule encoding another entity such as, for example, a signal peptide. It may also comprise additional 5 nucleotide sequence information fused, linked or otherwise associated with it either at the 3' or 5' terminal portions or at both the 3' and 5' terminal portions. The nucleic acid molecule may also be part of a vector, such as an expression vector.

The derivatives of the nucleic acid molecule of the present invention include oligonucleotides, 10 PCR primers, antisense molecules, molecules suitable for use in co-suppression and fusion nucleic acid molecules. Ribozymes and DNAzymes are also contemplated by the present invention directed to *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* or their mRNAs. Derivatives and homologs of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* are conveniently 15 encompassed by those nucleotide sequences capable of hybridizing to one or more of SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5 or SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9 or their complementary forms under low stringency conditions.

20 Derivatives include fragments, parts, portions, mutants, variants and mimetics from natural, synthetic or recombinant sources including fusion nucleic acid molecules. Derivatives may be derived from insertion, deletion or substitution of nucleotides.

Another aspect of the present invention provides an isolated expression product or a 25 derivative, homolog, analog or mimetic thereof which is produced in larger or lesser amounts in red gastrocnemius muscle in obese animals compared to lean animals or in fed (including re-fed) compared to fasted animals or in animals under exercise trained compared to control conditions.

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An expression product, as indicated above, may be RNA or protein. Insofar as the product is a protein, derivatives include amino acid insertional derivatives such as amino and/or carboxylic terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues 5 are introduced into a predetermined site in a protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterized by the removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. An example of substitutional amino acid variants are conservative amino 10 acid substitutions. Conservative amino acid substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. Additions to amino acid sequences include fusions with other peptides, polypeptides or proteins.

15 Chemical and functional equivalents of protein forms of the expression products AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 or AGT-710 should be understood as molecules exhibiting any one or more of the functional activities of these molecules and may be derived from any source such as being chemically synthesized or 20 identified *via* screening processes such as natural product screening or screening of chemical libraries.

The derivatives include fragments having particular epitopes or parts of the entire protein fused to peptides, polypeptides or other proteinaceous or non-proteinaceous molecules.

25 Reference herein to AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 or AGT-710 includes reference to isolated or purified naturally occurring AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 or AGT-710 as well as any derivatives, homologs, analogs and mimetics thereof. Derivatives

include parts, fragments and portions of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 or AGT-710 as well as single and multiple amino acid substitutions, deletions and/or additions to AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 when the expression products are proteins.

5 A derivative of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 or AGT-710 is conveniently encompassed by molecules encoded by a nucleotide sequence capable of hybridizing to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5 or SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9 under low stringency conditions.

10

Other derivatives of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 include chemical analogs. Analogs of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 contemplated herein include, but are not limited to, modifications to side chains, incorporation of unnatural

15 amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogs.

Examples of side chain modifications contemplated by the present invention include

20 modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH₄; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylolation of amino groups with 2, 4, 6-trinitrobenzene sulfonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH₄.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

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The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitization, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic

5 acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

10

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetrannitromethane to form a 3-nitrotyrosine derivative.

15

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis

20 include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 4.

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TABLE 4
Codes for non-conventional amino acids

	Non-conventional amino acid	Code	Non-conventional amino acid	Code
5	α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
	α -amino- α -methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
10	aminocyclopropane- carboxylate	Cpro	L-N-methyleasparagine	Nmasn
	aaminoisobutyric acid	Aib	L-N-methyleaspartic acid	Nmasp
	aminonorbornyl- carboxylate	Norb	L-N-methylcysteine	Nmcys
			L-N-methylglutamine	Nmgln
			L-N-methylglutamic acid	Nmglu
15	cyclohexylalanine	Chexa	L-Nmethylhistidine	Nmhis
	cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
	D-alanine	Dal	L-N-methylleucine	Nmleu
	D-arginine	Darg	L-N-methyllysine	Nmlys
	D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
20	D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
	D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
	D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
	D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
	D-isoleucine	Dile	L-N-methylproline	Nmpro
25	D-leucine	Dleu	L-N-methylserine	Nmser
	D-lysine	Dlys	L-N-methylthreonine	Nmthr
	D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
	D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
30	D-proline	Dpro	L-N-methylethylglycine	Nmetg
	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug

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	D-threonine	Dthr	L-norleucine	Nle
	D-tryptophan	Dtrp	L-norvaline	Nva
	D-tyrosine	Dtyr	α -methyl-aminoisobutyrate	Maib
	D-valine	Dval	α -methyl- γ -aminobutyrate	Mgabu
5	D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
	D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpen
	D- α -methylasparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
	D- α -methylaspartate	Dmasp	α -methylpenicillamine	Mpen
	D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
10	D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
	D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmaabu
	D- α -methylleucine	Dmleu	α -naphthylalanine	Anap
	D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
15	D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
	D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
20	D- α -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
	D- α -methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
	D- α -methylvaline	Dmval	N-cyclododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
25	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
30	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr

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	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
	D-N-methylleucine	Dnmleu	N-(3-indolylethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu
5	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
10	N-(2-methylpropyl)glycine	Nieu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyla-naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ -aminobutyric acid	Gabu	N-(<i>p</i> -hydroxyphenyl)glycine	Nhtyr
15	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L- α -methylalanine	Mala
	L- α -methylarginine	Marg	L- α -methylasparagine	Masn
	L- α -methylaspartate	Masp	L- α -methyl- <i>t</i> -butylglycine	Mtbug
20	L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- α -methylglutamine	Mgln	L- α -methylglutamate	Mglu
	L- α -methylhistidine	Mhis	L- α -methylhomophenylalanine	Mhphe
	L- α -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L- α -methylleucine	Mleu	L- α -methyllysine	Mlys
25	L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
	L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
	L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
	L- α -methylserine	Mser	L- α -methylthreonine	Mthr
	L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr
30	L- α -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe

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N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl- ethylamino)cyclopropane	Nmbc		

5

Crosslinkers can be used, for example, to stabilize 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional
10 reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α -methylamino acids; introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogs
15 by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

All such modifications may also be useful in stabilizing the AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 molecule for
20 use in *in vivo* administration protocols or for diagnostic purposes.

As stated above, the expression product may be a RNA or protein.

The term "protein" should be understood to encompass peptides, polypeptides and
25 proteins. The protein may be glycosylated or unglycosylated and/or may contain a range of other molecules fused, linked, bound or otherwise associated to the protein such as amino acids, lipids, carbohydrates or other peptides, polypeptides or proteins. Reference hereinafter to a "protein" includes a protein comprising a sequence of amino acids as well as a protein associated with other molecules such as amino acids, lipids, carbohydrates or
30 other peptides, polypeptides or proteins.

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In a particularly preferred embodiment, the expression product is encoded by a sequence of nucleotides comprising SEQ ID NO:1 or a derivative, homolog or analog thereof including a nucleotide sequence having at least about 30% similarity to SEQ ID NO:1 or a nucleotide sequence capable of hybridizing to SEQ ID NO:1 or its complementary form under low stringency conditions.

5

In another particularly preferred embodiment, the expression product is encoded by a sequence of nucleotides comprising SEQ ID NO:2 or a derivative, homolog or analog thereof including a nucleotide sequence having at least about 30% similarity to SEQ ID NO:2 or a nucleotide sequence capable of hybridizing to SEQ ID NO:2 or its complementary form under low stringency conditions.

10

In still another particularly preferred embodiment, the expression product is encoded by a sequence of nucleotides comprising SEQ ID NO:3 or a derivative homolog or analog thereof including a nucleotide sequence having at least about 30% similarity to SEQ ID NO:3 or a nucleotide sequence capable of hybridizing to SEQ ID NO:3 or their complementary form under low stringency conditions.

15

In yet another particularly preferred embodiment, the expression product is encoded by a sequence of nucleotides comprising SEQ ID NO:4 or a derivative homolog or analog thereof including a nucleotide sequence having at least about 30% similarity to SEQ ID NO:4 or a nucleotide sequence capable of hybridizing to SEQ ID NO:4 or their complementary form under low stringency conditions.

20

25 In another particularly preferred embodiment, the expression product is encoded by a sequence of nucleotides comprising SEQ ID NO:5 or a derivative homolog or analog thereof including a nucleotide sequence having at least about 30% similarity to SEQ ID NO:5 or a nucleotide sequence capable of hybridizing to SEQ ID NO:5 or its complementary form under low stringency conditions.

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In still another particularly preferred embodiment, the expression product is encoded by a sequence of nucleotides comprising SEQ ID NO:6 or a derivative homolog or analog thereof including a nucleotide sequence having at least about 30% similarity to SEQ ID NO:6 or a nucleotide sequence capable of hybridizing to SEQ ID NO:6 or its
5 complementary form under low stringency conditions.

In a further particularly preferred embodiment, the expression product is encoded by a sequence of nucleotides comprising SEQ ID NO:7 or a derivative homolog or analog thereof including a nucleotide sequence having at least about 30% similarity to SEQ ID
10 NO:7 or a nucleotide sequence capable of hybridizing to SEQ ID NO:7 or its complementary form under low stringency conditions.

In still yet another particularly preferred embodiment, the expression product is encoded by a sequence of nucleotides comprising SEQ ID NO:8 or a derivative homolog or analog
15 thereof including a nucleotide sequence having at least about 30% similarity to SEQ ID NO:8 or a nucleotide sequence capable of hybridizing to SEQ ID NO:8 or its complementary form under low stringency conditions.

In yet another particularly preferred embodiment, the expression product is encoded by a sequence of nucleotides comprising SEQ ID NO:9 or a derivative homolog or analog
20 thereof including a nucleotide sequence having at least about 30% similarity to SEQ ID NO:9 or a nucleotide sequence capable of hybridizing to SEQ ID NO:9 or its complementary form under low stringency conditions.
25 Higher similarities are also contemplated by the present invention such as greater than 40% or 50% or 60% or 70% or 80% or 90% or 95% or 96% or 97% or 98% or 99% or above.

Another aspect of the present invention is directed to an isolated expression product selected from the list consisting of:-

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(i) an mRNA or protein encoded by a novel nucleic acid molecule which molecule is differentially expressed in red gastrocnemius muscle from *P. obesus* animals under fed or fasting conditions or in exercise trained and control animals or a derivative, homolog, analog, chemical equivalent or mimetic thereof;

5 (ii) an mRNA or protein encoded by a novel nucleic acid molecule which molecule is differentially expressed in red gastrocnemius muscle from *P. obesus* animals under fed or fasting conditions or in exercise trained and control animals or a derivative, homolog, analog, chemical equivalent or mimetic thereof;

10 (iii) AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 or AGT-710 or a derivative, homolog, analog, chemical equivalent or mimetic thereof;

15 (iv) a protein encoded by a nucleotide sequence comprising SEQ ID NO:1 or a derivative, homolog or analog thereof or a sequence encoding an amino acid sequence having at least about 30% similarity to this sequence or a derivative, homolog, analog, chemical equivalent or mimetic of said protein;

20 (vi) a protein encoded by a nucleotide sequence substantially comprising SEQ ID NO:2 or a derivative, homolog or analog thereof or a sequence encoding an amino acid sequence having at least about 30% similarity to this sequence or a derivative, homolog, analog, chemical equivalent or mimetic of said protein;

25 (vii) a protein encoded by a nucleotide sequence substantially comprising SEQ ID NO:3 or a derivative, homolog or analog thereof or a sequence encoding an amino acid sequence having at least about 30% similarity to these sequences or a derivative, homolog, analog, chemical equivalent or mimetic of said protein;

30 (viii) a protein comprising an amino acid sequence substantially as set forth in SEQ ID NO:4 or a derivative, homolog or analog thereof or a sequence encoding an amino

acid sequence having at least about 30% similarity to these sequences or a derivative, homolog, analog, chemical equivalent or mimetic of said protein;

- (ix) a protein encoded by a nucleotide sequence substantially comprising SEQ ID NO:5
5 or a derivative, homolog or analog thereof or a sequence encoding an amino acid sequence having at least about 30% similarity to this sequence or a derivative, homolog, analog, chemical equivalent or mimetic of said protein;
- (x) a protein encoded by a nucleotide sequence substantially comprising SEQ ID NO:6
10 or a derivative, homolog or analog thereof or a sequence encoding an amino acid sequence having at least about 30% similarity to this sequence or a derivative, homolog, analog, chemical equivalent or mimetic of said protein;
- (xi) a protein encoded by a nucleotide sequence substantially comprising SEQ ID NO:7
15 or a derivative, homolog or analog thereof or a sequence encoding an amino acid sequence having at least about 30% similarity to this sequence or a derivative, homolog, analog, chemical equivalent or mimetic of said protein;
- (xii) a protein encoded by a nucleotide sequence substantially comprising SEQ ID NO:8
20 or a derivative, homolog or analog thereof or a sequence encoding an amino acid sequence having at least about 30% similarity to this sequence or a derivative, homolog, analog, chemical equivalent or mimetic of said protein;
- (xiii) a protein encoded by a nucleotide sequence substantially comprising SEQ ID NO:9
25 or a derivative, homolog or analog thereof or a sequence encoding an amino acid sequence having at least about 30% similarity to this sequence or a derivative, homolog, analog, chemical equivalent or mimetic of said protein;
- (xiv) a protein encoded by a nucleic acid molecule capable of hybridizing to a nucleotide
30 sequence comprising SEQ ID NO:1 or its complementary form or a derivative, homolog or analog thereof under low stringency conditions;

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- (xv) a protein encoded by a nucleic acid molecule capable of hybridizing to a nucleotide sequence comprising SEQ ID NO:2 or its complementary form or a derivative, homolog or analog thereof under low stringency conditions;
- 5 (xvi) a protein encoded by a nucleic acid molecule capable of hybridizing to a nucleotide sequence comprising SEQ ID NO:3 or their complementary forms or a derivative, homolog or analog thereof under low stringency conditions;
- 10 (xvii) protein encoded by a nucleic acid molecule capable of hybridizing to a nucleotide sequence comprising SEQ ID NO:4 or their complementary forms or a derivative, homolog or analog thereof under low stringency conditions;
- 15 (xviii) a protein encoded by a nucleic acid molecule capable of hybridizing to a nucleotide sequence comprising SEQ ID NO:5 or its complementary form or a derivative, homolog or analog thereof under low stringency conditions;
- 20 (xix) a protein encoded by a nucleic acid molecule capable of hybridizing to a nucleotide sequence comprising SEQ ID NO:6 or its complementary form or a derivative, homolog or analog thereof under low stringency conditions;
- 25 (xx) a protein encoded by a nucleic acid molecule capable of hybridizing to a nucleotide sequence comprising SEQ ID NO:7 or its complementary form or a derivative, homolog or analog thereof under low stringency conditions;
- (xxi) a protein encoded by a nucleic acid molecule capable of hybridizing to a nucleotide sequence comprising SEQ ID NO:8 or its complementary form or a derivative, homolog or analog thereof under low stringency conditions; and

(xxii) a protein encoded by a nucleic acid molecule capable of hybridizing to a nucleotide sequence comprising SEQ ID NO:9 or its complementary form or a derivative, homolog or analog thereof under low stringency conditions.

5 The protein of the present invention is preferably in isolated form. By "isolated" is meant a protein having undergone at least one purification step and this is conveniently defined, for example, by a composition comprising at least about 10% subject protein, preferably at least about 20%, more preferably at least about 30%, still more preferably at least about 40-50%, even still more preferably at least about 60-70%, yet even still more preferably
10 80-90% or greater of subject protein relative to other components as determined by molecular weight, amino acid sequence or other convenient means. The protein of the present invention may also be considered, in a preferred embodiment, to be biologically pure.

15 Without limiting the theory or mode of action of the present invention, the expression of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* is thought to relate to regulation of body weight and glucose homeostasis. Modulation of expression of these genes is thought *inter alia* to regulate energy balance *via* effects on energy intake and also effects on carbohydrate/fat metabolism. The energy
20 intake effects are likely to be mediated *via* the central nervous system but peripheral effects on the metabolism of both carbohydrate and fat are possible. The expression of these genes may also be regulated by fasting and feeding. Accordingly, regulating the expression and/or activity of these genes or their expression products provides a mechanism for regulating both body weight and energy metabolism, including
25 carbohydrate and fat metabolism.

The identification of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* permits the generation of a range of therapeutic molecules capable of modulating expression of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*,
30 *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* or modulating the activity of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710*.

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Modulators contemplated by the present invention include agonists and antagonists of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* expression. Antagonists of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* expression include antisense molecules, 5 ribozymes and co-suppression molecules (including any molecules which induce RNAi). Agonists include molecules which increase promoter activity or which interfere with negative regulatory mechanisms. Antagonists of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* include antibodies and inhibitor peptide fragments. All such molecules may first need to be modified to enable 10 such molecules to penetrate cell membranes. Alternatively, viral agents may be employed to introduce genetic elements to modulate expression of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710*. In so far as *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* act in association with other genes such as the *ob* gene which encodes leptin, the 15 therapeutic molecules may target *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* and *ob* genes or their translation products.

The present invention contemplates, therefore, a method for modulating expression of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and 20 *AGT-710* in a mammal, said method comprising contacting the *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* gene with an effective amount of a modulator of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* expression for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of *AGT-701*, 25 *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710*.

For example, a nucleic acid molecule encoding *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* or a derivative or homolog thereof may be introduced into a cell to enhance the ability of that cell to produce *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710*, conversely, *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-* 30

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709 and *AGT-710* sense and/or antisense sequences such as oligonucleotides may be introduced to decrease expression of the genes at the level of transcription, post-transcription or translation. Sense sequences preferably encode hair pin RNA molecules or double-stranded RNA molecules.

5

Another aspect of the present invention contemplates a method of modulating activity of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 in a mammal, said method comprising administering to said mammal a modulating effective amount of a molecule for a time and under conditions sufficient to

10 increase or decrease AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 activity. The molecule may be a proteinaceous molecule or a chemical entity and may also be a derivative of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 or its ligand.

15 Modulating levels of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* expression or AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-710 activity or function is important in the treatment of a range of conditions such as obesity, anorexia, energy imbalance, diabetes, metabolic syndrome, dyslipidemia, hypertension and insulin

20 resistance. It may also be useful in the agricultural industry to assist in the generation of leaner animals, or where required, more obese animals. Accordingly, mammals contemplated by the present invention include but are not limited to humans, primates, livestock animals (e.g. pigs, sheep, cows, horses, donkeys), laboratory test animals (e.g. mice, rats, guinea pigs, hamsters, rabbits), companion animals (e.g. dogs, cats) and

25 captured wild animals (e.g. foxes, kangaroos, deer). A particularly preferred host is a human, primate or livestock animal.

Accordingly, the present invention contemplates therapeutic and prophylactic use of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-710 expression products or *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*,

AGT-707, AGT-708, AGT-709 and AGT-710 genetic mutants and/or agonists or antagonists agents thereof.

The present invention contemplates, therefore, a method of modulating expression of *AGT-5 701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-710* in a mammal, said method comprising contacting the *AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-710* genes with an effective amount of an agent for a time and under conditions sufficient to up-regulate, down-regulate or otherwise module expression of *AGT-701, AGT-702, AGT-704, AGT-10 705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710*.

Another aspect of the present invention contemplates a method of modulating activity of *AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-710* in a subject, said method comprising administering to said subject a *15 modulating effective amount of an agent for a time and under conditions sufficient to increase or decrease AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-710 activity or function.*

Modulation of activity by the administration of an agent to a mammal can be achieved by *20 one of several techniques, including, but in no way limited to, introducing into a mammal a proteinaceous or non-proteinaceous molecule which:*

- (i) modulates expression of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-710;*
- (ii) functions as an antagonist of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-710; and/or*
- (iii) functions as an agonist of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-710.*

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The molecules which may be administered to a mammal in accordance with the present invention may also be linked to a targeting means such as a monoclonal antibody, which provides specific delivery of these molecules to the target cells.

5 A further aspect of the present invention relates to the use of the invention in relation to mammalian disease conditions. For example, the present invention is particularly useful in a therapeutic or prophylactic treatment of obesity, anorexia, diabetes or energy imbalance.

Accordingly, another aspect of the present invention relates to a method of treating a
10 mammal suffering from a condition characterized by one or more symptoms of obesity, anorexia, diabetes and/or energy imbalance, said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707,
15 AGT-708, AGT-709 and/or AGT-710 or sufficient to modulate the activity of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-
710.

In another aspect, the present invention relates to a method of treating a mammal suffering from a disease condition characterized by one or more symptoms of obesity, anorexia,
20 diabetes or energy imbalance, said method comprising administering to said mammal an effective amount of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-710 or AGT-701, AGT-702, AGT-704, AGT-705, AGT-
706, AGT-707, AGT-708, AGT-709 and/or AGT-710.
25 An agent includes proteinaceous or non-proteinaceous molecules such as antibodies, natural products, chemical entities or nucleic acid molecules (including antisense molecules, sense molecules, ribozymes, ds-RNA molecules or DNA-targeting molecules).

An "effective amount" means an amount necessary at least partly to attain the desired
30 immune response (e.g. against AGT-701, AGT-702, AGT-704, AGT-705, AGT-706,

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AGT-707, AGT-708, AGT-709 or AGT-710) or to delay the onset or inhibit progression or halt altogether the onset or progression of a particular condition.

In accordance with these methods, AGT-701, AGT-702, AGT-704, AGT-705, AGT-706,

5 AGT-707, AGT-708, AGT-709 and/or AGT-710 or *AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709* and/or *AGT-710* or agents capable of modulating the expression or activity of said molecules may be co-administered with one or more other compounds or other molecules. By "co-administered" is meant simultaneous administration in the same formulation or in two different formulations *via* the same or

10 different routes or sequential administration by the same or different routes. By "sequential" administration is meant a time difference of from seconds, minutes, hours or days between the administration of the two types of molecules. These molecules may be administered in any order.

15 In yet another aspect, the present invention relates to the use of an agent capable of modulating the expression of *AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709* and/or *AGT-710* or a derivative, homolog or analog thereof in the manufacture of a medicament for the treatment of a condition characterized by obesity, anorexia, weight maintenance, diabetes and/or energy imbalance.

20 In still yet another aspect, the present invention relates to the use of an agent capable of modulating the activity of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-710 or a derivative, homolog, analog, chemical equivalent or mimetic thereof in the manufacture of a medicament for the treatment of a

25 condition characterized by obesity, anorexia, weight maintenance, diabetes and/or energy imbalance.

A further aspect of the present invention relates to the use of *AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709* and/or *AGT-710* or derivative, homolog or analog thereof or AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and/or AGT-710 or derivative, homolog, analog, chemical

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equivalent or mimetic thereof in the manufacture of a medicament for the treatment of a condition characterized by obesity, anorexia, weight maintenance, diabetes and/or energy imbalance.

5 Still yet another aspect of the present invention relates to agents for use in modulating the expression of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and/or *AGT-710* or a derivative, homolog or analog thereof.

A further aspect relates to agents for use in modulating *AGT-701*, *AGT-702*, *AGT-704*,
10 *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and/or *AGT-710* activity or a derivative, homolog, analog, chemical equivalent or mimetic thereof.

Still another aspect of the present invention relates to *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and/or *AGT-710* or derivative, homolog or
15 analog thereof or *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and/or *AGT-710* or derivative, homolog, analog, chemical equivalent or mimetic thereof for use in treating a condition characterized by one or more symptoms of obesity, anorexia, weight maintenance, diabetes and/or energy imbalance.
20 In a related aspect of the present invention, the mammal undergoing treatment may be a human or an animal in need of therapeutic or prophylactic treatment.

Accordingly, the present invention contemplates in one embodiment a composition comprising a modulator of *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*,
25 *AGT-708*, *AGT-709* and *AGT-710* expression or *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* activity and one or more pharmaceutically acceptable carriers and/or diluents. In another embodiment, the composition comprises *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* or a derivative, homolog, analog or mimetic thereof
30 and one or more pharmaceutically acceptable carriers and/or diluents. The compositions may also comprise leptin or modulations of leptin activity or *ob* expression.

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For brevity, all such components of such a composition are referred to as "active components".

- 5 The compositions of active components in a form suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of
- 10 microorganisms such as bacteria and fungi.

The carrier can be a solvent or other medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils.

15

- 15 The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable
- 20 compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

- 25 Sterile injectable solutions are prepared by incorporating the active components in the required amount in the appropriate solvent with optionally other ingredients, as required, followed by sterilization by, for example, filter sterilization, irradiation or other convenient means. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

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When *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* or *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* are suitably protected, they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed

5 in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active

10 compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1

15 µg and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the

20 like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For

25 instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active

30 compound may be incorporated into sustained-release preparations and formulations.

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Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is

5 incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for
10 ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by
15 and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

20 The principal active component may be compounded for convenient and effective administration in sufficient amounts with a suitable pharmaceutically acceptable carrier in dosage unit form. A unit dosage form can, for example, contain the principal active component in amounts ranging from 0.5 µg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 µg to about 2000 mg/ml of
25 carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

In general terms, effective amounts of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706,
30 AGT-707, AGT-708, AGT-709 and AGT-710 or AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 will range from 0.01

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ng/kg/body weight to above 10,000 mg/kg/body weight. Alternative amounts range from 0.1 ng/kg/body weight to above 1000 mg/kg/body weight. The active ingredients may be administered per minute, hour, day, week, month or year depending on the condition being treated. The route of administration may vary and includes intravenous, intraperitoneal, 5 sub-cutaneous, intramuscular, intranasal, via suppository, via infusion, via drip, orally or via other convenient means.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable 10 of modulating AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 expression or AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 activity. The vector may, for example, be a viral vector.

15 Still another aspect of the present invention is directed to antibodies to AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 and their derivatives and homologs insofar as AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 are proteins. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies 20 to AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 or may be specifically raised to AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 or derivatives or homologs thereof. In the case of the latter, AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 or their derivatives or homologs may first 25 need to be associated with a carrier molecule. The antibodies and/or recombinant AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 or their derivatives of the present invention are particularly useful as therapeutic or diagnostic agents. An antibody "to" a molecule includes an antibody specific for said molecule.

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AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 and their derivatives can be used to screen for naturally occurring antibodies to AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 which may occur in certain autoimmune diseases. Alternatively, specific

5 antibodies can be used to screen for AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA.

Antibodies to AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708,

10 AGT-709 and AGT-710 of the present invention may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to the *AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710* or may be specifically raised to these gene products. In the case of the latter, the AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 protein may need first
15 to be associated with a carrier molecule. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies. The antibodies of this aspect of the present invention are particularly useful for immunotherapy and may also be used as a
20 diagnostic tool or as a means for purifying AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710.

For example, specific antibodies can be used to screen for AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 proteins. The latter

25 would be important, for example, as a means for screening for levels of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 in a cell extract or other biological fluid or purifying AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 made by recombinant means from culture supernatant fluid. Techniques for the assays contemplated herein are known
30 in the art and include, for example, sandwich assays and ELISA.

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It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An 5 antibody as contemplated herein includes any antibody specific to any region of AGT-119, AGT-120, AGT-121, AGT-122, AGT-422, AGT-123 and AGT-504.

Both polyclonal and monoclonal antibodies are obtainable by immunization with the enzyme or protein and either type is utilizable for immunoassays. The methods of 10 obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of AGT-119, AGT-120, AGT-121, AGT-122, AGT-422, AGT-123 and AGT-504, or antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoabsorbent techniques. Although antibodies 15 produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The 20 preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art. (See, for example, Douillard and Hoffman, Basic Facts about Hybridomas, in *Compendium of Immunology* Vol. II, ed. by Schwartz, 1981; Kohler and Milstein, *Nature* 256: 495-499, 25 1975; Kohler and Milstein, *European Journal of Immunology* 6: 511-519, 1976.)

Another aspect of the present invention contemplates a method for detecting AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 or a derivative or homolog thereof in a biological sample from a subject, said method 30 comprising contacting said biological sample with an antibody specific for AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710

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or their antigenic derivatives or homologs for a time and under conditions sufficient for a complex to form, and then detecting said complex.

The presence of the complex is indicative of the presence of AGT-701, AGT-702, AGT-
5 704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710. This assay may
be quantitated or semi-quantitated to determine a propensity to develop obesity or other
conditions or to monitor a therapeutic regimen.

The presence of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-
10 708, AGT-709 and AGT-710 may be accomplished in a number of ways such as by
Western blotting and ELISA procedures. A wide range of immunoassay techniques are
available as can be seen by reference to U.S. Patent Nos. 4,016,043, 4,424,279 and
4,018,653. These, of course, include both single-site and two-site or "sandwich" assays of
the non-competitive types, as well as in the traditional competitive binding assays. These
15 assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is
20 immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 complex, a second antibody specific to the AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and
25 AGT-710, labeled with a reporter molecule capable of producing a detectable signal, is then added and incubated, allowing time sufficient for the formation of another complex of antibody-AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710-labeled antibody. Any unreacted material is washed away, and the presence of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708,
30 AGT-709 and AGT-710 is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal,

or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will

5 be readily apparent. In accordance with the present invention, the sample is one which might contain AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710 including cell extract, tissue biopsy or possibly serum, saliva, mucosal secretions, lymph, tissue fluid and respiratory fluid. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation

10 fluid and supernatant fluid such as from a cell culture.

The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other

15 surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex to the solid surface which is then washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes or overnight if

20 more convenient) and under suitable conditions (e.g. from room temperature to about 37°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710. The second antibody is linked to a reporter

25 molecule which is used to indicate the binding of the second antibody to AGT-701, AGT-702, AGT-704, AGT-705, AGT-706, AGT-707, AGT-708, AGT-709 and AGT-710.

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be

30 labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the

antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

5 By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

10

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase,

15 β -galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted

20 above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically,

25 to give an indication of the amount of hapten which was present in the sample. A "reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

30 Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody

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absorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent-labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is 5 then exposed to the light of the appropriate wavelength. The fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

10

The present invention also contemplates genetic assays such as involving, for example, PCR analysis to detect *AGT-701*, *AGT-702*, *AGT-704*, *AGT-705*, *AGT-706*, *AGT-707*, *AGT-708*, *AGT-709* and *AGT-710* or their derivatives.

15 Real-time PCR is also particularly useful for assaying for particular genetic molecules.

The present invention is further described by the following non-limiting Examples.

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EXAMPLE 1

Psammomys obesus

In the following examples, *Psammomys obesus* rats were used for differential expression studies under different conditions. The rats are divided into three groups, based on metabolic phenotype, as follows:-

10	Group A animals : lean
	Group B animals : obese, non-diabetic
	Group C animals : obese, diabetic.

EXAMPLE 2

Sequence of Psammomys obesus AGT-701

15 AGT-701 was identified using microarray analysis of red gastrocnemius muscle in exercise trained and control *P. obesus*.

The nucleotide sequence is as follows:-

20	GGACATTTTCAGCCATGAGGAGCTTCTGGAAACTCGGAGTTGAACAG AAATATAGGAATAATAATCACGCCAGGCTCCTAACCTGGAGAACATTGAGCTG TACTGGAACAGCTACAACAACCAGCCGAGACCTGAACCTCGAGCGAGGTGGT GAGATGACCCTCAAGTGCCCTGTGATGCTGGTAGGAGACCAAGCGCCT CATGAGGATGCCGTGGTAGTGTAACTCAAACACTGGACCCCACACAGACC TCGTTCCCTCAAGATGGCTGATTCTGGAGGTCAAGCCACAGCTGACCCAGCCA GGCAAGCTGACTGAGGCTTCAAGTACTNCTGCAAGGCATGGGCTACATG GCCTCCTCCTGCATGACTCGCCTATCGAGGTCTGCACGGCATTTGACC AGCCGAGCATTGAT [SEQ ID NO:1]
25	

30

EXAMPLE 3

AGT-701 sequence homology

N-myc downstream-regulated gene 2 (NDRG2)

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EXAMPLE 4

AGT-701 gene expression

NDRG2 is a cytosolic protein of 371 amino acids with a molecular mass of 40.7 kDa

5 (Zhou *et al.*, *Genomics* 73(1): 86-97, 2001) and is encoded by a 2.4 kb mRNA. By radiation hybrid analysis, Kalaydjieva *et al.* (*Am. J. Hum. Genet.* 67(1): 47-58, 2000) mapped the NDRG2 gene to chromosome 14q11.2. No information currently exists on the function of NDRG2. The human and mouse NDRG2 proteins are 92% identical (Zhou *et al.*, 2001). Rat NDRG2 was recently identified and has approximately 90% homology to

10 the mouse and human protein (Boulkroun *et al.*, *J. Biol. Chem.* 277(35): 31506-31515, 2002).

NDRG2 is part of the NDRG family, which includes NDRG1, NDRG3 and NDRG4. At the amino acid level, the four members share 53-65% identity (Kalaydjieva *et al.*, 2000; Zhou *et al.*, 2001). NDRG1 and NDRG3 belong to one subfamily and NDRG2 and NDRG4 to another. The N- and C-terminal regions are the most divergent regions between the four NDRG proteins, however the C-terminal five aa residues, Met-Glu-Val-Ser-Cys-COOH [SEQ ID NO:12] are conserved in all human and mouse proteins. There are three tandem repeats of GTRSRSHTSE [SEQ ID NO:13] in the C-terminal region of NDRG1

20 which are not present in NDRG2, NDRG3 and NDRG4.

All members of the NDRG family are cytosolic proteins (Qu *et al.*, *Mol. Cell. Biochem.* 229(1-2): 35-44, 2002). Each of the four proteins contains an α/β hydrolase fold which is common to a number of hydrolytic enzymes, suggesting that NDRG2 may have an enzymatic function (Boulkroun *et al.*, 2002). Hydrolases are enzymes that catalyze the hydrolysis of various bonds, e.g. C-O, C-N, C-C, phosphoric anhydride bonds, etc. Hydrolysis is the rupture of one or more chemical bonds by reaction with, and involving the addition of, the elements of water.

30 NDRG1 was the first of the family identified and has been shown to be involved in stress responses, hormone responses, cell growth, and differentiation (Zhou *et al.*, 2001 and

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references therein). NDRG1 gene expression is up-regulated by many agents, such as reducing agents, tunicamycin, lysophosphatidylcholine, okadaic acid, calcium ionophore, DNA damaging agents, nickel compounds, forskolin, and androgens. The gene is also up-regulated during cell differentiation, in response to hypoxia, and at certain stages of the cell cycle in a p53-dependent manner. Over-expression of NDRG1 in tumor cells decreases the proliferation rate, enhances differentiation, and suppresses the metastatic potency of the cancer cells. In contrast, NDRG1 is repressed by N-myc and c-myc and in many tumor cells. A nonsense mutation in the NDRG1 gene is causative for hereditary motor and sensory neuropathy-Lom (HMSNL), a severe peripheral neuropathy characterized by Schwann cell dysfunction and progressive axonal loss in the peripheral nervous system. This suggests that NDRG1 functions in the peripheral nervous system necessary for axonal survival.

After the identification of NDRG1 in human and mouse, two other members of the family were then identified in mouse, Ndr2 and Ndr3 (Okuda and Kondoh, *Biochem. Biophys. Res. Commun.* 266(1): 208-215, 1999). In contrast to NDRG1, Ndr2 and Ndr3 were not under negative regulation by N-myc. Their expression during mouse development indicates that the three members of the family are under distinct spatio-temporal regulations, implying that genes of the NDRG family probably have tissue-dependent allotments of the possibly related functions (Okuda and Kondoh, 1999). Using the novel mouse sequences of Ndr2 and Ndr3 to search the human genome databases, Kalaydjieva *et al.*, 2000 identified the homologous human genes, which they referred as NDRG2 and NDRG3. Zhou *et al.*, 2001 cloned NDRG3 and NDRG4, studied the human NDRG gene family and further characterized NDRG4.

NDRG1, NDRG2 and NDRG3 are all expressed in a wide variety of tissues. NDRG2 is most highly expressed in adult skeletal muscle, brain and heart (Zhou *et al.*, 2001; Qu *et al.*, 2002), and NDRG3 is most highly expressed in brain and testis (Zhou *et al.*, 2001; Qu *et al.*, 2002). NDRG4 is specifically expressed in brain and heart (Zhou *et al.*, 2001; Qu *et al.*, 2002).

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Zhou *et al.*, 2001 identified two forms of NDRG2, with and without a 14 amino acid insertion in the N-terminal region, located after amino acid number 25. This insertion was also present in mouse Ndr2. They designated the form with the insertion NDRG2 and the form without the insertion NDRG2^{var}. Rat NDRG2 has four isoforms (Boulkroun *et al.*, 5 2002). They differ in their 5'UTR sequence, which are either 87 or 50 nucleotides in length, and the presence or absence of the 42 base pairs (14 amino acids) insertion in the coding sequence at the same site as in human and mouse. The proteins are 357 or 371 amino acids in length. The insertion corresponds to the inclusion of exon 3 of the human DRG2 gene, suggesting an alternative splicing event. Sequences highly homologous to the 10 2 rat NDRG2 5'UTR were found on different exons on the human NDRG2 gene, indicating that they might correspond to alternative 5' untranslated exons (Boulkroun *et al.*, 2002). This genomic organization strongly suggests the presence of alternative promoters which could direct expression of NDRG2 in a tissue specific and developmentally regulated manner.

15 NDRG2 has 34% identity to *Drosophila* MESK2, a component of the Ras pathway (Boulkroun *et al.*, 2002). Ras is an upstream regulator of phosphatidylinositol 3 kinase, and it may be hypothesized that NDRG2 may affect skeletal muscle insulin signalling through that pathway.

20 A Glucocorticoid Responsive Element (GRE) half-site (TGTTCT) is present in the human NDRG2 promoter (Boulkroun *et al.*, 2002). However, the glucocorticoid dexamethasone did not alter NDRG2 expression in RCCD2 cells, a rat kidney cortical collecting duct cell line, while the glucocorticoid-regulated gene sgk was induced.

25 In conclusion, NDRG2 belongs to a family of genes putatively involved in growth arrest and induction of cell differentiation, the Ras pathway, and the peripheral nervous system. Although it has strong homology to NDRG1, it is not under negative regulation by N-myc. NDRG2 is a cytosolic protein, probably with an enzymatic function.

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EXAMPLE 5

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Red Gastrocnemius muscle: exercise training

AGT-701 gene expression in skeletal muscle of *P. obesus* increased with exercise training, and was negatively correlated with blood glucose and the change in blood glucose after 5 training. AGT-701 expression also correlated positively with energy expenditure (Figures 1 to 4).

EXAMPLE 6

Red Gastrocnemius muscle: fasting (24 h)

10

AGT-701 expression was significantly lower in C fed *P. obesus*, compared to A fed animals and significantly higher in B fasted *P. obesus* compared to A fasted and C fasted animals. AGT-701 expression negatively correlated with body fat, body weight and blood glucose in the fed *P. obesus* (Figures 5 to 9).

15

EXAMPLE 7

Sequence of Psammomys obesus AGT-702

AGT-702 was identified using microarray analysis of red gastrocnemius muscle in exercise 20 trained and control *P. obesus*.

The nucleotide sequence is as follows:-

25 GCTGGTACCGGTCCGGATTCCCGGGATATCGTCGACCCACGCCTCCGGTG
 GTGGAGAACGATCGCTCCTGCCGTGGTCACATTGAACGTATCGCAAACCTT
 CCTTCTCGAAGAGGGAGGTGCCAGTGGCGAGTGGTCCGGATTATCGTG
 TCTGAGGATGGACTGATTGTGACCAATGCTCACGTGGTACCAACAAAAAC
 AGGGTCAAGGTTGAGCTGAAGAACGGAGCAACCTATGAAGCTAAAATCAAG
 GATGTGGATGAAAAGGCAGACATCGCACTTATCAAAATTGACCACCAAGGGA
30 AAGCTGCCAGTCTTGCTGCTGGCCGCTCCTCAGAGCTCGACCAGGAGAG
 TTTGTGGTCGCCATCGGAAGCCCCTTTCCCTCAAAACACAGTCACCACT
 GGGATCGTCAGTACCAACCCAGCGAGGGGGCAAAGAGCTGGGGC [SEQ ID
NO : 2]

35

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EXAMPLE 8

AGT-702 sequence homology

Protease, serine 11 (PRSS11).

5

EXAMPLE 9

Gene expression as measured by SYBR green real time PCR:

Red Gastrocnemius muscle; Exercise Training

10 AGT-702 gene expression in skeletal muscle of *P. obesus* increased after exercise training and was negatively correlated with body weight in exercise trained *P. obesus*. AGT-702 expression was positively correlated with energy expenditure, and negatively correlated with blood glucose and the training induced change in blood glucose in all *P. obesus* (Figures 10-14).

15

EXAMPLE 10

Sequence of AGT-704 Psammomys obesus

AGT-704 was identified using microarray analysis of red gastrocnemius muscle in exercise
20 trained and control *P. obesus*.

The nucleotide sequence is as follows:-

25 TGACATTTCTTCACCTCTTATGATAGCTGATATACTAAATCTTTAT
ACAGAAATGTCAGTACTTGAAACAAATTCAAAACACATTGGTTATTAACCTT
TTGGCTCATGCATGGTTATTAGGTTCAAATTACCTGATTCTATCTATAT
TTACTTTAAAATGTGTGGTTCTCATTAAAAGTAAAACACTAAACAGTG
CTTTGGAATTCTAAGCTACTAATTGTTGATAGATAACAGCCTGTGTCTAG
TAAAATAGTTTGTGGGTGTGGGTCTATCTTCCATGAAAAAGTGGGAGG
30 TGTAAGTTAGTTGGTAGTGCTAATAGTTAAATTATATAAAATAAGAA
TGAGCATTGGTATCTGTATGAAAGGCCCTAAATCAAATGATTATCCAT
AATCAATCTTATTCTTGTAAACCAAGGGCACTCATGGTTAA
GTGTGCTGAGATAGAAAAG [SEQ ID NO:3]

35

EXAMPLE 11

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AGT-704 sequence homology

Mus musculus RIKEN cDNA 1200009K13 gene (1200009K13Rik), mRNA. There are no human matches with the *P. obesus* sequence. However when BLASTing the mouse
5 sequence NM_025814 against the NR database, it matches strongly to Homo sapiens CGI-55 protein mRNA and Homo sapiens PAI-1 mRNA-binding protein (PAI-RBP1). These are the same gene with the LocusLink and Unigene cluster calling it PAI-RBP1.

EXAMPLE 12

10 ***AGT-704 gene expression***

PAI-RBP1 is a 387 amino acid protein (with additional six and/or 15 amino acids insert in some variants) that plays a role in regulation of mRNA stability. Regulation of mRNA stability is an important component of the regulation of gene expression and is known to
15 have a significant role in normal physiology and development.

The PAI-RBP1 protein binds to an A-rich region in the 3' 134 nucleotides of the PAI-1 mRNA. This 134 nucleotides region is able to confer cyclic nucleotide regulation of mRNA stability and is, therefore, called the CRS (cyclic nucleotide-responsive sequence).
20 The PAI-1 CRS includes a 75 nucleotide U-rich region at its 5' end and a 24 nucleotides A-rich region at its 3' end. Mutation of the A-rich portion reduces binding by PAI-RBP1 and eliminates cyclic nucleotide regulation of mRNA decay.

The amino acid sequence of PAI-RBP1 includes an RGG box at amino acid 343-359, as
25 well as an Arg-rich (amino acid 126-137) and an RG-rich (amino acid 163-184) motif, which places it in the general category with RNA-binding proteins, even though it does not have other RNA binding motifs such as an RNA recognition motif (RRM) or K-homology (KH) domain. The potential protein kinase A phosphorylation site (RKES) at serine 74 is also important given that this protein could be regulated by cyclic nucleotides.

PAI-RBP1 includes blocks of sequence that are highly conserved in a number of metazoans including mammals, birds, *Drosophila* and *Arabidopsis*. Thus, PAI-RBP1 identifies a family of proteins with a previously unidentified domain that may define a new RNA-binding motif.

5

PAI-RBP1 has four splice variants, from two alternative splice sites, in both human and rat. An insertion of six amino acids after position 202 is found in some transcripts, and an insertion of 15 amino acids after amino acid 226 is found in some, both with or without the six amino acid insert.

10

PAI-RBP1 mRNA is expressed in a wide variety of tissues suggesting that it has a more general biological role involving regulation of mRNA stability or processes requiring interaction with RNA.

15 Plasmin is a broad spectrum protease. It is the major fibrinolytic enzyme in blood and also participates in a number of physiological and pathological processes involving localized proteolysis. Plasminogen is converted to plasmin by plasminogen activators (PAs), which are serine proteases and hydrolyze one peptide bond of plasminogen. Plasminogen activator activity is regulated by plasminogen activator inhibitor 1 (PAI-1). It is the mRNA
20 of PAI-1 that PAI-1-RBP1 binds to. PAI-1 expression is also regulated by growth factors, cytokines and hormones including agents that regulate cAMP levels.

PAI-1 is consistently elevated in obesity and type 2 diabetes (Mertens and Van Gaal, *Obes. Rev.* 3(2): 85-101, 2002). There is a strong positive correlation between this elevated PAI-1 and the degree of hyperinsulinemia. Both modest and substantial weight loss have been found to significantly reduce PAI-1 levels. Recently it has been demonstrated that the adipocyte itself is able to produce PAI-1. Only the abdominal fat, not femoral subcutaneous fat, PAI-1 gene expression contributes to increases in plasma PAI-1 in obesity (Mavri *et al.*, *Diabetologia* 44(11): 2025-2031, 2001). Adipose tissue also
30 produces several effector molecules that can up regulate PAI-1. These molecules include transforming growth factor β , TNF α , angiotensin II and interleukin 6. Insulin stimulates

- 69 -

PAI-1 gene expression but glucose transport and PAI-1 gene expression are mediated by different insulin signaling pathways (Samad *et al.*, *Mol. Med.* 6(8): 680-692, 2000). The disturbances in the haemostatic and fibrinolytic systems in part explains why obese and type 2 diabetic patients are at risk for the development of cardiovascular diseases.

- 5 Increased PAI-1 levels in the blood vessel wall decreases local fibrinolysis which may elevate thrombus formation and the evolution of atherosclerotic plaques (Pandolfi *et al.*, *Arterioscler Thomb. Vasc. Biol.* 21(8): 1378-1382, 2001). Chronic inflammation has emerged as a new risk factor for the development of type 2 diabetes. Elevated levels of acute-phase proteins and PAI-1 predict type 2 diabetes independent of insulin resistance
- 10 and other known risk factors for diabetes (Festa *et al.*, *SJ*(4): 1131-1137, 2002).

A 4G/5G polymorphism in the PAI-1 promoter is strongly linked to obesity, and a markedly increased risk for obesity is associated with the 4G allele in its homozygous form (Hoffstedt *et al.*, *Diabetologia* 45(4): 584-587, 2002). Regular exercise has been shown to

- 15 be effective for controlling elevated PAI-1 levels in subjects homozygous for the 4G allele (Vaisanen *et al.*, *Thomb. Haemost.* 82(3): 1117-1120, 1999).

EXAMPLE 13

Gene expression as measured by SYBR green real time PCR:

- 20 *Red Gastrocnemius muscle; Exercise Training*

AGT-704 gene expression in skeletal muscle of *P. obesus* increased with exercise training. AGT-704 gene expression negatively correlated with blood glucose and positively correlated with energy expenditure in *P. obesus* (Figures 15 to 17).

- 25

EXAMPLE 14

Red Gastrocnemius muscle; Fasting (24 hr)

There were no differences in AGT-704 gene expression between the fed or the fasted groups of *P. obesus*, although there was a trend towards decreased expression in C fed *P.*

- 30

- 70 -

obesus compared to A fed ($p=0.08$). However, AGT-704 gene expression showed a significant negative correlation with blood glucose in fed *P. obesus* (Figures 18 and 19).

EXAMPLE 15

5 *Sequence of AGT-705 Psammomys obesus*

AGT-705 was identified using microarray analysis of red gastrocnemius muscle in exercise trained and control *P. obesus*.

10 The nucleotide sequence is as follows:-

15	CGCAGATACTGCAGAGCTGACGCATTCTTGCCCTGGCATCTCAGCTTGCTA GGTGTCTCATCTCGCTCTGCGCTCGTGGCCTCCTCCAAGGCCTCCAGTCT CCTTAAGAAAACCTCAAGACCTGGAAAGCTACGATGCGAGCTTGATGCCGC TACCCCTAGCAGGCTATGGACTCCTGAGGGTCTCGGACTGTTGACACCCA TTCCGATCCGCATCCCTCCAAGCTGATAAGCCCAGGACCCCTAGGGCGGGGT GCCAGACTCATGTGTGACGCCCTGCACTGAAACCCCATTCCCAGTGTGGT TGCTTCTTGCTGGCTTGGCCATTGATACCACGAAGGATGACGATGC TAGTTATGCAGCAGCCAACACACCTCCCCCAACTCTCCGCTGTCACTGGT GGGCCCCACTGTCCAGGAAGCAGGTGTCCGGAACTGACATCTGGAGCAGA GGGGCCATGAGAGGGTGTGTATCCTGCCAGAAAGCAGCTGGACCACGACG CTCCCAAGATGAACCCACTGTATACAGAGGCATCATGGGAGTTGTTATGTC AGGAGCATTCTAGACCCACGTGTACTTGAGCGTGGAAAGACAGAAAGANANG CGCAGAGACTGGGCACCTGATCTGCTCACCATGATCGCCTGCACGGGTCT 25 CATCCAGTTCTGCCCTAGGCTACAGTGGCGGTGTCCACGGGCTTGCCATT CAAACGTGCTCTCAGACCCAGATGGGCTCACCACGTGAGGAGAACCTTTCA CTTGGTGGGTATGCAGAGGGAAAGGGTCTCGACTCCAGAGACCTGGAGTCC AAAGTTGTTTTTTTATTACAGTAATTATTTATTATTTTATTATT AGTTATGAGTCCAAAGTTAATACCATTCAAGCTACTGCTGTCTGCT 30 TAGAGCCACAGCATGCAATGTGGCACCAAGGCATCCTGCTCACAGTT CACACTGTGGGAACAGGCATCCTGTTCTACAGATTAGCGCGAGGGAAAC CAGAAATATTAAACACGCAGGGTTGTCTCTCCAAGGGAGAGGCACATACC CTGTTTCTCCGAAGGCTGGAGGGAGGTGTTGATATCCTGGCTACC TCTGTGCAATCTGTAGGCCATGTCCTTAAGATGTAGCTGTCAGTCGGTAGT GGAGCCGGAGCCGTCACTCAGTAGATTGGGGTTGTGGCATGCGCCTTAAC 35 TCCATTAAATTCCAGCACTCTAGTGGTTGGTACAGCAGCAGCAGCGG TTGCAGTGGCCGGGAAGTCCTGAAGACCAAGCTTCTACCCAGCACTCAG GCAGCAAAGGCAGGGTGGATTCTCTGAGTTGAGGCCGGCTGGTCTACAGA GTGAGTCCAGNCNAGCCAGGGCTACAACAGAGAAANCCTCTATTGAAAAA 40 ATAAATAAATTATAAAAAAAAAAGGTGTATGTGTCTGTACTTTACA AAGAATGTTGATGCTTAAGCTTTGTGCACNCAAGAAAATTGTTAACT
----	--

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5

GGTGTCACTCCTGAAGTTGAACCAGCACTTAGCCNGGCGTGGTGGCGC
ACGCCTGTAATCCCAGCCCTCGGGAGGCAGAGGCAGGTGGATCTCTGAGTT
AGAGGCCAGCCTGGTCTACAGAGTGAGTCCAGGACAGCCAGGATTACACAG
AGAAAACCCGTCTCAAAAATGTAAAATAAATTAAAATAAGTTGAACCAA
CAGTGTTTACTGAGTCGTGAAACAGATTACCTTTGCTTCTCTTGA
TCATTATTCTACTGTGGTGTCAAGCAGAGACCCCTCCAGCAGGTGGCCAACG
TGAGAGTCTCAAGCCGAGAAGGTAAGAATTAAAAAAAAAAAAAAA
[SEQ ID NO:4]

10

EXAMPLE 16

AGT-705 sequence homology

The full clone sequence of AGT-705 matches a mouse mRNA clone BC030414 (not full length mRNA) but no human sequences on the GenBank database.

15

EXAMPLE 17

Gene expression as measured by SYBR green real time PCR:

Red Gastrocnemius muscle; Exercise Training

20 AGT-705 expression was not different between the exercise trained and the control *P. obesus*, although there was a tendency for expression to increase with exercise training (Figure 20).

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EXAMPLE 18

Red Gastrocnemius muscle; Fasting (24 hr)

In this study, there was no difference in AGT-705 expression between the fed *P. obesus*,
however, there was a trend towards increased expression in the C fed *P. obesus*. AGT-705
expression was significantly higher in the B fasted group when compared with the A fasted
group and the C fasted group and gene expression was negatively correlated with blood
glucose in the fasted *P. obesus* (Figures 21 to 23).

10

EXAMPLE 19

Sequence of AGT-706 Psammomys obesus

AGT-706 was identified using microarray analysis of red gastrocnemius muscle in exercise trained and control *P. obesus*.

15

The nucleotide sequence is as follows:-

GCCAAATGGATTCTGTTGAGATGTTGGATGAAAACAAGTATCCACTGTT
TACCAACTTGACGAAAAATCTCAACTGAGGTTGGCTGTTAAAAAAAAA
ATTCACTGTGGCCTCTGTCCTTAATTGTCGAAACCATTGTGACTGTTAC
TGCTCAAAGTATCGTACTGTTCATAGTAACATACATCAGAATTGCACCGC
TGCTGTTGGAAAAGCCAATAAAGAAACCCCCAGACTGCTGCTCAGCAAAT
GTTAATAAAAGTGTGCGCACCGTAGGCCGTGTCACCCAGTCACCAAGCAGC
GTCCCTTGTCTGCGAGTGGCTGTGGGTGTGATTNACCACCTCAGAGGTG
CACAGCACCTGCTTGNCCCTTAAGTGTGNGTCAGAAGACAAGCAGCTTC
TCGGTAACCAACAACCTGCTTTCGGAGCTCAGTGTGTTAGGCTGTTACT
GAATCANATATGTAACTCAGCACACATAAGCGAAGAGAGATTGGCTGC
ACTGGCAAGAGTGAACCAAATTACTTCTATTTTAAAGGCAGATCATA
TTTAAGCATATAAGTAATTATGGATATAAATTGTTGGATATTATTTA
GTCTGAATATTGTTTAAATTATTACATGTGTTCTCTATGTCTTATC
TCTGGAATAACGATGCCATTAACCACATGCCATATGTTTGAAAGTGG
GTGNAACAGAGGAAAAGTCATCCTCTGGTCTTGACTCCCTTCCTCA
ACTACATGATAAGTCTATCAATAAAGCATTGACCTCAGCAGGGCAGAA
GCCTGNAAGTTAGAAAACTCATTGACCACAGTAGACAATTGATTCTTA
GAAATAAGAAGTGTGAGAACGAGCTGCTGNGCTGAGCAGGGGATGTAACCA
AGTCCAGATGCACCAACGTGAAGAGGCTNTAGCAAAATATGTTGCCT
CTCACCCCTGCACATGTTCTAGATGCTTAAACAGCCACATGGCCCGC

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TGCGAGGACCTCGTAATGTTGTTGTTGTTGTTAAAGGAGTT
CTCACAAAGCGTACAAGTGCAGCACTGAAAGTGGCTGAGGCCACAGTCCT
CAGCACCCAAGTCTNTCCGCAAGCAGCCAAGCTGGTGTGTCGGGTGN
5 GTATGTCTGTGCTCAGTGCAGCTGGTGTGTCGGTATATTAT
GTGCCCAAGTGTGGGCGANAGCTGACCCANGCTGGACACACTTCTT
TTNGNCTTCGAGTTACTGGTGATNCAGNTAAAATAATTAAATTAAATT
AAAGACTT [SEQ ID NO:5]

EXAMPLE 20

10 *AGT-706 sequence homology*

Human hypothetical protein FLJ20069 and mouse Ahi-1 (also called mouse 1700015F03).

EXAMPLE 21

15 *AGT-706 gene expression*

The Ahi-1 locus was initially identified as a common helper provirus integration site in Abelson pre-B-cell lymphomas and shown to be closely linked to the c-myb proto-oncogene. Proviral integration within the Ahi-1 region has also been shown in thymomas 20 of T cell origin.

Jiang *et al.* (*J. Virol.* 76(18): 9046-9059, 2002) identified the murine gene (Ahi-1) targeted by these provirus insertional mutations. The Ahi-1 cDNA encodes a 1,047-amino-acid protein. The predicted Ahi-1 protein is a modular protein that exhibits several features of a 25 signaling molecule. It contains one SH3 motif and seven WD40 repeats. The Ahi-1 gene is conserved in mammals and encodes two major RNA species of 5 and 4.2 kb and several other shorter splicing variants. The Ahi-1 gene is expressed in mouse embryos and in several organs of the mouse and rat, notably at high levels in the brain and testes.

30 The Ahi-1 proviral insertions were found at the 3' end of the gene, in an inverse transcriptional orientation, with most of them located around and downstream of the last exon, whereas another insertion was within intron 22. In addition, another previously identified provirus insertion site, Mis-2, was found to map within the 16th intron of the Ahi-1 gene. In tumor cells harboring insertional mutations in Ahi-1, truncated Ahi-1/viral

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fused transcripts were identified, including some splicing variants with deletion of the SH3 domain.

In summary, Ahi-1 encodes a protein that exhibits several features of a signaling molecule
5 and is targeted by provirus insertion. Ahi-1 may play an important role in signal transduction in normal cells and may be involved in tumor development, possibly in cooperation with other oncogenes (such as v-abl and c-myc) or with a tumor suppressor gene (Nfl).

10

EXAMPLE 22

*Gene expression as measured by SYBR green real time PCR:
Red Gastrocnemius muscle; Exercise Training*

AGT-706 gene expression increased in skeletal muscle of *P. obesus* after exercise training.
15 In addition, AGT-706 gene expression was negatively correlated with blood glucose and positively correlated with insulin and energy expenditure in *P. obesus* (Figures 24 to 27).

EXAMPLE 23

Red Gastrocnemius muscle; Fasting (24 hr)

20

In this study, AGT-706 gene expression was significantly higher in the B fasted and C fasted groups, when compared to the A fasted group. AGT-706 gene expression was negatively correlated with blood glucose in fed *P. obesus*, and positively correlated with insulin in fasted *P. obesus* (Figures 28 to 30).

25

EXAMPLE 24

Sequence of AGT-707 Psammomys obesus

AGT-707 was identified using microarray analysis of red gastrocnemius muscle in exercise
30 trained and control *P. obesus*.

- 75 -

The nucleotide sequence is as follows:-

5 GTNGAAGCNTAGGAGTTGAGGATGCGCCGATGTCGAGCCGCTGGAACCC
 ACGCTTAGCAATATCATCGAGCAGCGCAGCCTTAAGTGGATCTCGTCGGG
 GGCAAGGGTGGCGTTGGTAAGACCACCTGCAGCTGCAGCCTGGCGGTCCAG
 CTGTCTAAGGGACGTGAGAGTGTCTAATCATTCCACAGACCCAGCTCAC
 AACATCTCAGATGCATTGACCAGAAGTTCTCCAAGGTGCCTACCAAGGTC
 AAAGGCTATGACAACCTCTTGCTATGGAGATAGACCCGAGCCTGGCGTG
 GCAGAGCTCCCTGATGAAGTTCTCGAGGAAGACAACATGCTGAGCATGGG
 10 CAAGAAGATGATGCAGGAGGCCATGAGCGCCTT [SEQ ID NO:6]

EXAMPLE 25

AGT-707 sequence homology

15 ASNA1: Human homolog of bacterial *arsA* arsenite transporter, ATP-binding.

EXAMPLE 26

AGT-707 gene expression

20 ASNA1 is the human homolog of the bacterial *arsA* gene. In *E. coli*, ArsA ATPase is the catalytic component of a multi-subunit oxyanion pump that is responsible for resistance to arsenicals and antimoniais. The *E. coli* *ars* operon contains two regulatory (*arsR* and *arsD*) and three structural genes (*arsA*, *B* and *C*). The *arsA* gene codes for an oxyanion ATPase that associates with the protein encoded for by *arsB*, the channel-forming transmembrane 25 protein. Together, the two proteins transport arsenite and antimonite out of the cells across the plasma membrane.

Human ASNA1 encodes a 332-amino acid polypeptide having an N-terminal ATP-binding cassette (ABC) domain and a C-terminal domain of unknown function (Kurdi-Haidar *et al.*, *Genomics* 36: 486-491, 1996). The protein sequence is highly homologous throughout both domains to hypothetical *arsA* proteins of *C. elegans* and yeast. Southern blot analysis indicated the existence of two closely related ARSA genes in the human genome. The existence of a second human ARSA protein was further supported by Western blot analysis, which demonstrated that anti-ARSA1 antibodies identify two proteins of 37 and

- 76 -

42 kD. Kurdi-Haider *et al.*, 1996 expressed ASNA1 and found that the resulting 37-kD protein had ATPase activity.

Northern blot analysis revealed that the ASNA1 gene is expressed in a variety of tissues,
5 with highest expression in the cardiac and skeletal muscle (Kurdi-Haider *et al.*, 1996). Immunohistochemical analysis of normal human tissues detected ASNA1 only in the epithelial cells of the liver, kidney, and stomach wall, in the adrenal medulla, in the islet cells of the pancreas, in the red pulp of the spleen, and in cardiac and skeletal muscle (Kurdi-Haidar *et al.*, *J. Histochem. Cytochem.* 46: 1243-1248, 1998d). In skeletal muscle
10 the fibers were strongly positive. Interestingly, ASNA1 levels were markedly increased in breast fibroadenomas and carcinomas.

ASNA1 shows a cytoplasmic, perinuclear, and nucleolar distribution (Kurdi-Haidar *et al.*, *J. Biol. Chem.* 273: 22173-22176, 1998a; Kurdi-Haidar *et al.*, *J. Cell. Biochem.* 71: 1-10,
15 1998b). Since the nuclear membrane and the nucleolus were enriched for ASNA1, with no detectable protein in the nucleoplasm, suggests that the nuclear ASNA1 is bound and does not diffuse freely. The cytoplasmic ASNA1 is soluble. The ASNA1 at the nuclear membrane was associated with invaginations into the nucleus in interphase cells. These results and the fact that it is not found in the plasma membrane suggest that ASNA1 is a
20 paralog rather than an ortholog of ArsA and that it probably plays a different role in human cells than does the ArsA protein in bacteria. In human cells it appears to play a role in the nucleocytoplasmic transport of a nucleolar component.

Kurdi-Haider *et al.*, 1998a characterized purified recombinant ASNA1. They determined
25 that the ATPase activity increases in the presence of sodium arsenite (but not antimonite) and that Vmax rather than ATP affinity is enhanced. Human ASNA1 is an arsenite-stimulated rather than an arsenite-dependent ATPase, and has significant basal ATPase activity even in the absence of oxyanions. Kurdi-Haider *et al.*, 1998a found that the active species is likely a dimer or tetramer.

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Kurdi-Haidar *et al.*, *Somat. Cell Molec. Genet.* 24: 307-311, 1998c mapped the ASNA1 gene to chromosome 19q13.3 and determined that it contains four exons and spans 6 kb.

EXAMPLE 27

5

Mouse ASNA1 gene

Mouse Asna1 encodes a 348-amino acid protein sharing 27% and 99% identity with the *E. coli* and human proteins, respectively (Bhattacharjee *et al.*, *Gene* 272: 291-299, 2001). Northern blot analysis detected a 1.3-kb transcript in mouse at highest levels in kidney and
10 testis, moderate levels in brain, liver, lung, and skin, low levels in heart, small intestine, spleen, stomach, and thymus, and negligible levels in skeletal muscle. Bhattacharjee *et al.*, 2001 mapped the mouse Asna1 gene to the C3-D1 region of chromosome 8, and determined that it consists of seven exons spanning over 7 kb.

15

EXAMPLE 28

Gene expression as measured by SYBR green real time PCR:

Red Gastrocnemius muscle; Exercise Training

AGT-707 gene expression in skeletal muscle of *P. obesus* increased with exercise training.
20 There were no correlations between gene expression and other phenotypic variables (Figure 31).

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EXAMPLE 29

Red Gastrocnemius muscle; Fasting (24 hr)

There were no differences in AGT-707 gene expression between the fed groups or the
 5 fasted groups, although there was a trend for increased expression in group A compared to
 group B and C *P. obesus* in the fed state ($p=0.075$ and $p=0.055$, respectively). AGT-707
 gene expression was negatively correlated with body weight in the fed *P. obesus* (Figures
 32 and 33).

10

EXAMPLE 30

Sequence of AGT-708 Psammomys obesus

AGT-708 was identified using microarray analysis of red gastrocnemius muscle in exercise
 15 trained and control *P. obesus*.

The nucleotide sequence is as follows:-

20	AAAATTTACAAATGAGTGTGAATTGCATTCTGATATAATAATTATCACCC CACCACACTTTACTGACACTGTTGATGGCCTATGCTGTGTTTCACATCA CAATTCTTGATGGAAAAATTCTGTGGCCTGTGTAACCCCTCTGGTCAGT ATTATGAAACCAACTATCTTGGTATAAATAAGGTTCCGGTAAGATGCC AGGGTTCATGAGTATGGCACAAATAACAGAGGACAGGGAGGCCTCACGACG AAGGAGGCCCGTAAGTGGCCTGGAGGGCACAGATGCAGTTCCAGGTCAAGAA
25	AAGAGCAGCTTTCAACAGGCAGTCGTGGGTATGATGGAAACTCAGCCT GTCTCTGTAGTTATGGACAGCGTGGCAGGTGACTGTGCCACATCTCCTA TACAGTGCTTTTACTGACTGGAAGTACGTGAATCTCACTTAGTCCC CAACTGGACGTTCTGGAAAAACAAAGCAAATGTTAAAGTATGTCTTCT GGATATAGGCCAGNAGNAATACATTAAGAACATGAGAGGCCTGCTTGATC
30	TCAGCCATTGGAGGCTAGAAAAAAATTGAAAGGAACCTTCCTGTTGATAGA CTCAAAGCCGTGAACAGAACGCCTTGGCCTGTTCAAGACAATCTCTGGTA ATCTACTGACAATATCCAACAGTTCGATGTCCTGTTAACTACCCTGGT AGCTTTCTGTGGATTGAAGTTCAATTAAAGCTGTGGAATTCAAAC
35	GAATTACACGTGCATTTGTAAGAGTTCAGAACCCAGTGCTGAGTCTGTGTGG CAGGTTTTTTCACCGCGTGATATACTATTACAAATGCATGTGGTGCCATG CTTGTCTTCAAATATATAAGTAGTAGTGCTAAATGGATAAGTCATATGGAGCTT TTGATTTAG [SEQ ID NO: 7]

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EXAMPLE 31

AGT-708 sequence homology

Protein kinase inhibitor α (PKI α).

5

EXAMPLE 32

AGT-708 gene expression

cAMP-dependent protein kinases co-ordinate cellular responses to hormones and

10 neurotransmitters by altering processes such as cell division, membrane permeability and transcription. Most of the effects of cAMP in the eukaryotic cell are mediated through the phosphorylation of target proteins on serine or threonine residues by the cAMP-dependent protein kinase (EC 2.7.1.37). The inactive cAMP-dependent protein kinase is a tetramer composed of two regulatory and two catalytic subunits. The cooperative binding of four

15 molecules of cAMP dissociates the enzyme in a regulatory subunit dimer and two free active catalytic subunits. In the human, four different regulatory subunits (PRKAR1A, PRKAR1B, PRKAR2A, and PRKAR2B) and three catalytic subunits (PRKACA, PRKACB and PRKACG) have been identified.

20 Members of the cAMP-dependent protein kinase inhibitor family are specific and extremely potent competitive inhibitors of cAMP-dependent protein kinase activity. These proteins interact with the catalytic subunit of the enzyme after the cAMP-induced dissociation of its regulatory chains. The inhibitory site contains regions very similar to the hinge regions (sites that directly interact with the enzyme active site) and "pseudosubstrate site" of the regulatory chains; but unlike these chains, PKI does not contain cAMP-binding sites. The arginine residues within the inhibitory site are essential for inhibition and recognition of the enzyme active site.

25

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EXAMPLE 33

Cloning

Using the mouse protein kinase inhibitor-alpha to screen a human neuroblastoma cell line

5 cDNA library, Olsen and Uhler (*Molec. Endocr.* 5: 1246-1256, 1991) isolated clones encoding human PKIA. The deduced 75-amino acid PKIA protein shares 100% and 97% sequence identity with the rabbit and mouse homologs, respectively. Northern blot analysis detected major 4-kb and minor 2-kb PKIA transcripts in skeletal muscle. Using kinase assays with transfected COS cells, Olsen and Uhler, 1991 verified that the PKIA cDNA

10 produces a heat-stable inhibitor of protein kinase. Protein extracts inhibited both the α (601639) and β (176892) isoforms of the protein kinase catalytic subunit with equal efficacy. Using a transcriptional activation system, Olsen and Uhler, 1991 demonstrated that elimination of a conserved alternative translation start site in PKI increased the inhibitory activity of the PKI expression vector.

15

EXAMPLE 34

Gene expression as measured by SYBR green real time PCR:

Red Gastrocnemius muscle; Exercise Training

20 AGT-708 gene expression in skeletal muscle of *P. obesus* was negatively correlated with activity and the change in carbohydrate oxidation after exercise training, and was positively correlated with energy expenditure and the change in fat oxidation after exercise training when all animals were analyzed together. AGT-708 gene expression positively correlated with food intake and the change in fat oxidation and negatively correlated with

25 the change in carbohydrate oxidation in the exercise trained *P. obesus* (Figures 34 to 40).

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EXAMPLE 35

Red Gastrocnemius muscle; Fasting (24 hr)

AGT-708 gene expression was significantly higher in the B fasted and C fasted groups
 5 when compared to the A fasted group. AGT-708 gene expression positively correlated with blood glucose in fasted *P. obesus* (Figures 41 and 42).

EXAMPLE 36

Sequence of AGT-709 Psammomys obesus

10

AGT-709 was identified using microarray analysis of red gastrocnemius muscle in exercise trained and control *P. obesus*.

The nucleotide sequence is as follows:-

15

TGAGATAGCTACTCCATAAGCCTCTGAAGAGCAATAGCTAATTATTATTA
 CTGTAATTNTTTAAAGGCTTAAAGTGCCTGGGGGTCCCTGAAACTAA
 TTTCTACTTCTGGGATTCCCTGGATTCTTATAAGAGATGGTGACATGAC
 TAGGGAAATTCTTTTTAGTATGAAAATTGCCCTCAATACTTTCTCT
 20 TACTGGCATTGAATTATCACAGAGACAGAAAATTGGTAATTTTTTAATT
 CTAACTCTCCCAGAAAACCTCCCTTGCCCTAGTATTATGATGTGCTTA
 ACCATGGGAGGAGGGTGGGGGGAACTCATCAAGCTGCCAGTATTTG
 ATCTACAACCTGTAGCA [SEQ ID NO:8]

25

EXAMPLE 37

AGT-709 sequence homology

Human KIAA0663

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EXAMPLE 38

Gene expression as measured by SYBR green real time PCR:

Red Gastrocnemius muscle; Exercise Training

5 There was no difference in AGT-709 gene expression in exercise trained animals when compared to controls. A significant positive correlation between AGT-709 gene expression and body weight and energy expenditure was found when all animals were analyzed together. AGT-709 expression in exercise trained animals showed a positive association with body weight, and the change in glucose and in body weight after exercise training
10 (Figures 43 to 47).

EXAMPLE 39

Red Gastrocnemius muscle; Fasting (24 hr)

15 AGT-709 expression was significantly lower in the C fed *P. obesus* than the A fed *P. obesus*, although there were no differences in gene expression between the fasted groups. AGT-709 expression was negatively correlated with body weight and blood glucose in fed *P. obesus* (Figures 48 to 50).

20

EXAMPLE 40

Sequence of AGT-710 Psammomys obesus

AGT-710 was identified using microarray analysis of red gastrocnemius muscle in exercise trained and control *P. obesus*.

25

The nucleotide sequence is as follows:-

30

CCACACGCAGTCGGGTGGCTCTGCAGCACAATTAGGCCTTGGAGGGAGCTG
TGGTTGTCACCCTCTACAANATGGGCTTCCCCGAAGCGGNCAAGCTCCTTCA
GAACACACCCANATTCGGCTGCTCCCACCAAGCTCTGCAAGGGATGGATTCA
AGGCCAATCTTGTCTTAAGGAGATCGAGAAGAAAGCTTGAAGAGAGAAGGGG
AACAGTTCGTGAAGAAGATCGGTGGATTTCGCCTTCAAAGTGAAGGACG
GCCCTGGAGGCAAAGAAGCCACCTGGGTGGATGTGAAGAATGGCAAGG

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GATCCGTGCTTCCCAACTCAGATAAGAAGGCTGACTGCACAATCACCATGG
CCGACTCCGACTTGCTGGCTCTGATGACTGNAAAATGAACCCTC [SEQ
ID NO:9]

5

EXAMPLE 41

AGT-710 sequence homology

Sterol carrier protein 2 (SCP2)

10

EXAMPLE 42

AGT-710 gene expression

Sterol carrier protein 2 (SCP-2, SCP2) is also known as Nonspecific lipid-transfer protein, mitochondrial precursor (NSL-TP) and Sterol carrier protein X (SCP-X, SCPX) (SCP2
15 Genecard record, Genecards Website, Weizmann Instsitute of Science; Human SCP-2 (NLTP_HUMAN) record froM SWISS-PROT database, ExPasy Website). The SCP-2 gene is a fusion gene that has two initiation sites. The gene encodes two proteins: SCP-2 and SCP-X. Both proteins share the same C-terminal 13 kDa (123 aa) sequence. The SCP-
2 transcript encodes a 15 kDa (143 amino acid) pro-SCP-2 protein which is post-
20 translationally cleaved to form the mature 13 kDa SCP-2 protein. The longer isoform, SCP-X, is translated into a 58 kDa (547 amino acid) protein and is partially cleaved to form two proteins - the 13kDa SCP-2 and a 45 kDa (404 amino acid) protein (Gallegos *et al.*, *Prog. Lipid. Res.* 40(6): 498-563, 2001). The latter is a 3-ketoacyl-CoA-thiolase specific for branched chain acyl CoAs (Stolowich *et al.*, *Cell Mol. Life Sci.* 59(2): 193-212,
25 2002). In most tissues however, the majority of the 58 kDa protein remains intact (Stolowich *et al.*, 2002).

The 13 kDa SCP-2 binds a number of different ligands such as fatty acids, fatty acyl CoAs, cholesterol and phospholipids. It is thought that the 13 kDa SCP-2 facilitates the
30 intracellular transport of lipids such as cholesterol between membranes. SCP-2 has been shown to also interact with a number of other ligands, and other possible physiological functions are being examined (Gallegos *et al.*, 2001).

Related genes/proteins that also contain the C-terminal SCP-2 domain are:

DHB4 HUMAN: 17 β -hydroxysteroid dehydrogenase IV (DBH4 record from Pfam database of protein families Website, Sanger Centre; Gallegos *et al.*, 2001) (the C-terminal

5 SCP-2 domain is known to be required for peroxisomal import of this protein) and
UNC-24 protein from *C. elegans* (this protein consists of an N-terminal SPFH (or band 7) domain and a SCP-2-like C-terminal domain. The human homologue of this protein is stomatin-like protein (hSLP). Its function is unknown (Gallegos *et al.*, 2001; Barnes *et al.*, *J. Neurochem.* 67(1): 46-57, 1996).

10

EXAMPLE 43

Chromosomal Location and Gene Structure

The SCP-2 gene is located at chromosome 1p32 (Vesa *et al.*, *Hum. Molec. Genet.* 3: 341-15 346, 1994; SCP2 Genecard record, Genecards Website, Weizmann Institute of Science; Sterol Carrier Protein 2 record, OMIM Website). It consists of 16 exons and 15 introns in humans, mice, rats and chickens (Ohba *et al.*, *Genomics* 24: 370-274, 1994). The mouse homologue is found on chromosome 4 (Welch *et al.*, *Genome* 7: 624-625, 1996). There are 2 promoter regions that initiate at least 4 mRNA species (Stolowich *et al.*, 2002). Two 20 alternatively polyadenylated mRNA transcripts (mRNAs of 2.8kB and 2.2 kb) encode the 58 kDa SCP-X protein, and two alternatively polyadenylated transcripts (mRNAs of 1.5 kb and 0.9 kb) encode the 15 kDa pro-SCP-2 protein (Stolowich *et al.*, 2002). Another study (Yamamoto *et al.*, *Proc. Natl. Acad. Sci. USA* 88(2): 463-467, 1991) identified a further two transcripts in the liver (1.8 kB and 3.2 kb species) where the 1.8kb isoform was most 25 abundant. Little is known about the transcriptional regulation of the gene (Gallegos *et al.*, 2001].

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EXAMPLE 44

Structural Features

The secondary and tertiary structures for the 13 kDa molecule are available (Stolowich *et al.*, 2002; Szyperski *et al.*, *FEBS Lett.* 335(1): 18-26, 1993), however no structures are available for the 15 kDa, 45 kDa or 58 kDa molecules. At the protein sequence level the SCP-X protein consists of 3 domains (SCP-2 record from Pfam database of protein families Website, Sanger Center):

10 • Thiolase N-terminal domain (residues 11-240).
 • Thiolase C-terminal domain (residues 245-402).
 • SCP-2 at the C-terminal of the protein (residues 433-543).

15 The mature 13 kDa SCP-2 protein consists of only the SCP-2 domain. A tertiary structure is available for this molecule (Stolowich *et al.*, 2002). Functionally important structural elements that have been identified by elucidation of the structure are:-

20 • The N-terminal 32 residues form an amphipathic helix, one face of which is a membrane binding domain that binds to anionic phospholipids at membrane surfaces.
25 • The hydrophobic faces of the N-terminal amphipathic helices plus β -strands 4,5 and β -helix D form a ligand-binding cavity able to accommodate multiple types of lipids (fatty acids, acyl coAs, cholesterol, phospholipids, isoprenoids).
 • The C-terminus is highly hydrophobic and it is thought to form a hydrophobic cap that closes around the ligand upon binding.

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EXAMPLE 45

Putative biochemical function

Gallegos *et al.*, 2001 and Stolowich *et al.*, 2002 have reviewed the various functions of
5 SCP-2. In summary:-

- SCP-2 is believed to affect cholesterol synthesis - overexpression in mice of the rat
SCP-2 gene by adenoviral infection increased liver cholesterol levels by 70% and
decreased liver cholesterol synthesis by 60% (Zanlungo *et al.*, *Gastroenterology*
10 119(6): 1708-1719, 2000).
- SCP-2 (Fuichs *et al.*, *Biochem. J.* 336(1): 33-37, 1998; Publielli *et al.*, *Biochem. J.*
317(3): 681-687, 1996; Kawata *et al.*, *Clin. Chim. Acta* 197(3): 201-208, 1991; Fuchs
et al., *J. Biol. Chem.* 276(51): 48058-48065, 2001) and SCP-X (Bun-ya *et al.*, *J.*
15 *Biochem. (Tokyo)* 123(2): 347-352, 1998; Ferdinandusse *et al.*, *J. Lipid. Res.* 41(3):
336-342, 2000; Wanders *et al.*, *J. Inherit. Metab. Dis.* 21(3): 302-305, 1998)
participate in different aspects of bile acid synthesis.
- SCP-2 is believed to be involved in transport of cholesterol from ER to bile (Ito *et al.*,
20 *Gastroenterology* 110(5): 1619-1627, 1996).
- SCP-2 and possibly SCP-X and pro-SCP-2 are believed to be involved in
triacylglyceride formation (Seedorf *et al.*, *Genes Dev.* 12(8): 1189-1201, 1998;
Atshaves *et al.*, *J. Lipid Res.* 40(4): 610-622, 1999; Murphy and Schroeder, *Biochim.
25 Biophys. Acta* 1345(3): 283-292, 1997; Starodub *et al.*, *Am. J. Physiol. Cell Physiol.*
279(4): C1259-1269, 2000).
- SCP-2 and possible SCP-X and pro-SCP-2 are believed to participate in peroxisomal
fatty acid oxidation (Seedorf *et al.*, 1998; Schroeder *et al.*, *Biochemistry* 34(37):
30 11919-11927, 1995; Ossendorp *et al.*, *Arch. Biochem. Biophys.* 334(2): 251-260,
1996).

- SCP-2 is thought to facilitate cholesterol transport to mitochondria and be involved in regulating steroidogenesis (Yamamoto *et al.*, *Proc. Natl. Acad. Sci. USA* 88(2): 463-467, 1991; Yamamoto *Hokkaido Igaku Zasshi* 67: 839-848, 1992).

5

- The 58 kDa SCP-X is one of three 3-ketoacyl-CoA thiolases found in peroxisomes. SCP-X is believed to play an exclusive role in peroxisomal branched chain fatty acid oxidation (Antonenkov *et al.*, *J. Biol. Chem.* 272(41): 26023-26031, 1997; Antonenkov *et al.*, *Protein Expr. Purif.* 18(3): 249-256, 2000; Wanderrs *et al.*, *Biochem. Biophys. Res. Commun.* 236(3): 565-569, 1997) and in oxidation of the branched side chain of cholesterol to form bile acids (Fuchs *et al.*, 2001; Bun-ya *et al.*, 1998; Ferdinandusse *et al.*, 2000).

10

15

EXAMPLE 46

Scp2 Knockout Mice

scp2 null mice show a severe block at the level of thiolytic cleavage in pristanic acid β -oxidation and lack normal peroxisomal degradation of the cholesterol side chain in bile acid synthesis. The knockout mice show spontaneous peroxisome proliferation and increased mRNA levels of genes regulated by PPAR α . The *scp2* null phenotype is similar to that seen in acyl-CoA oxidase (ACO) null mice (Kannenberg *et al.*, *J. Biol. Chem.* 274(50): 35455-35460, 1999). The *scp2* null mice also have affected peroxisomal α -oxidation of phytanic acid (Atshaves *et al.*, 1999). Whether these phenotypes are secondary affects of the gene knockout has yet to be clarified (Seedorf *et al.*, *Biochim. Biophys. Acta* 1486(1): 45-54, 2000).

EXAMPLE 47

Tissue distribution

30 The human SCP2 gene was cloned from the liver (Yamamoto *et al.*, 1991). The protein has been found most highly expressed in liver, intestine, adrenal and kidney (Baum *et al.*, *J.*

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Lipid Res. 34(5): 729-739, 1993). It is also expressed in lung, brain, testes, ovary and heart, fibroblasts, and placenta (Human SCP-2 (NLTP_HUMAN) record from SWISS-PROT database, ExPasy Website). EST data from normal human tissues in Unigene indicate that the gene is expressed in bone marrow, brain, heart, skeletal muscle, liver, 5 pancreas, prostate, kidney, and lung (SCP2 Genecard record, Genecards Website, Weizmann Institute of Science).

EXAMPLE 48

Cellular Localization

10

The identical C-termini of both SCP-2 and SCP-X contain an SKL peroxisomal targeting signal, however, as much as half of the total SCP-2 is located outside the peroxisome. The SCP-2 N-terminal presequence in the pro-SCP-2 protein strongly modulates intracellular targeting coded for by the C-terminal peroxisomal signal sequence (Gallegos *et al.*, 2001; 15 Stolowich *et al.*, 2002). Other studies indicate that mammalian SCP-2 is found in the cytoplasm or the mitochondria and that SCP-X is found in peroxisomes (Baker *et al.*, *DNA Cell Biol.* 10(9): 695-698, 1991; Interpro database of proteins [<http://www.ebi.ac.uk/interpro/Ientry?ac=IPR003033>]).

20

EXAMPLE 49

Role in disease

SCP-2 levels are altered in diseases where lipid metabolism is abnormal, such as diabetes, Zellweger, Niemann Pick C (NPC) and atherosclerosis (Stolowich *et al.*, 2002). SCP-2 is 25 present in low levels in Zellweger syndrome but is not causal of this syndrome in which the cells are deficient in peroxisomes (SCP2 Genecard record, Genecards Website, Weizmann Institute of Science). Zellweger patients have no detectable 15 kDa pro-SCP-2 protein or the mature 13 kDa SCP-2 and are deficient in very long chain fatty acid oxidation (Wirtz, *Biochem. J.* 324(2): 353-360, 1997; van Heusden *et al.*, *J. Biol. Chem.* 30 265(7): 4105-4110, 1990).

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The SCP-X/SCP-2 gene was investigated as a candidate gene for infantile neuronal ceroid lipofuscinosis. However, despite the gene mapping to the same chromosomal location as markers for this disease, no association could be found between mutations in the SCP-2/SCP-X gene and the disease (Vesa *et al.*, 1994).

5

NPC1 disease is caused by a mutation in the NPC protein. In this disease cholesterol accumulates in liver lysosomes and the Golgi. This disease shows markedly reduced levels of hepatic 13 kDa SCP-2 as well as accumulation of lipids in lysosomes and Golgi (Roff *et al.*, *J. Biol. Chem.* 267(22): 15902-15908, 1992).

10

EXAMPLE 50

Relationship to obesity or diabetes

Streptozotocin-induced diabetes in rats decreased liver levels of SCP-2 by 60-90% and
15 ovarian levels by 60% (McLean *et al.*, *Biol. Reprod.* 55(1): 38-46, 1996). Reduced 13 kDa
SCP-2 expression in pregnant diabetic mice was associated with pregnancy loss (McLean
et al., 1996).

EXAMPLE 51

20 *Gene expression as measured by SYBR green real time PCR:*

Red Gastrocnemius muscle; Exercise Training

AGT-710 gene expression in skeletal muscle of *P. obesus* increased with exercise training.
Gene expression was positively correlated with energy expenditure when all *P. obesus*
25 were analysed together. Gene expression was negatively correlated with blood glucose and
activity when all *P. obesus* were analyzed together. Gene expression was positively
correlated with fat oxidation and the change in fat oxidation in exercise trained *P. obesus*
(Figures 48 to 53).

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EXAMPLE 52

Red Gastrocnemius muscle; Fasting (24 hr)

AGT-710 gene expression was significantly lower in C fed *P. obesus* when compared to A fed *P. obesus*. AGT-710 gene expression was negatively correlated with body weight and blood glucose in fed *P. obesus*. There were no differences in gene expression between the fasted groups, and no correlations with phenotypic variables (Figures 54 to 59).

EXAMPLE 53

cDNA microarray production

RNA extracted from *P. obesus* was used to generate a cDNA library in the pCMV-SPORT 6 Vector (Invitrogen Life Technologies). Individual cDNA clones were arrayed into 384 well plates (The Australian Genome Research Facility, Queensland, Australia). The clones were then PCR amplified using vector complimentary primers (SP6 5'ATT TAG GTG ACA CTA TAG 3' [SEQ ID NO:10]; T7: 5'-TAATACGACT CACTATAGGG-3' [SEQ ID NO:11]). PCR amplification of each clone was performed in a GeneAmp PCR System 9700 thermal cycler (PE Applied BioSystems, Sunnyvale, CA) for 35 cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec and extension at 72°C for 120 sec. A final extension step was performed at 72°C for 5 min. Products were visualized by TAE agarose gel (1.5% w/v) electrophoresis at 6V/cm for 90 min to ensure successful amplification had taken place.

PCR products were purified using the ArrayIt vacuum manifold system (TeleChem International, Sunnyvale, CA) and resuspended in 20 µL of 1X spotting solution (TeleChem) at a concentration of 0.5 mg/ml in 384 well plate format. 5 µL of the resuspended purified cDNA solution was transferred to 384 well uniplates (Whatman Inc, Clifton, USA). This cDNA was arrayed onto Super Amine Microarray Substrates (TeleChem) using a Chip Writer Pro robotic arrayer (Virtek, Toronto, Canada) fitted with 16 Stealth SMP-03 quill tipped microarray pins (Telechem). The distance between adjacent cDNA spots was 200 µM. Each pin drew 0.25 µL of cDNA and deposited approximately

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0.6 nL on each slide. Humidity was maintained between 55-65% during printing. Approximately 12,000 elements were printed per microarray. Spotted DNAs were allowed to dry overnight, after which the slides were washed and blocked as recommended by the manufacturer (TeleChem).

5

EXAMPLE 54

RNA Extraction

Total RNA was extracted from tissue in a two-step process utilising Trizol (Invitrogen Life Technologies, Carlsbad, USA) and RNeasy (Qiagen, Hilden, Germany) protocols. The tissue samples were lysed in 1.5 ml of Trizol (Invitrogen Life Technologies) and homogenised using a Ystral Homogeniser (model D-7801, Dottingen, Germany). 300 µL of chloroform was added to the homogenate, which was then mixed, transferred into a fresh 2 ml tube and incubated at room temperature for 3 min. The homogenates were then separated by centrifugation at 13 000xg for 15 min (4°C). Following centrifugation the aqueous supernatant was collected in 2 ml tubes and an equal volume of 70% v/v ethanol added, with the solution mixed by pipetting. 700 µL of the sample was then transferred via pipette into RNeasy kit mini spin columns (Qiagen) placed in 2 ml tubes (supplied) for purification. Initially the sample was centrifuged at 10 000xg for 20 sec. The flow-through was then poured back into the column and the centrifugation repeated. Further purification was performed according to manufacturer's instructions and the RNA was eluted using RNAase free water.

Following purification, total RNA integrity, quantity and concentration was assessed using the RNA 6000 Nano Assay (Agilent Technologies, Palo Alto, USA) with the Agilent 2100 Bioanalyser (Agilent Technologies) as per the manufacturer's instructions. This system utilises capillary electrophoresis to separate and detect nucleic acid fragments by size through the interconnected micro channels on a Nano chip (Agilent Technologies). Good quality RNA is signified by an electropherogram displaying a marker peak, and two ribosomal peaks of which the 18s band is at an approximate ratio of 1:2 to the 28s band.

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EXAMPLE 55

Indirect labeling of cDNA

Fluorescently labelled cDNA was prepared from 20 µg of total RNA using an indirect
5 labelling method. cDNA synthesis was performed in a 30 µL reaction containing 5 µg
oligo-dT primer, 400U SuperscriptII (Invitrogen), 1x first strand buffer, 0.01 M DTT, 0.5
mM of each dATP, dCTP and dGTP, 0.150 mM dTTP (Amersham, Buckinghamshire,
UK) and 0.2 mM aminoallyl-dUTP (Sigma, St. Louis, MO). Synthesis was conducted in a
GeneAmp PCR System 9700 (PE Applied Systems) at 42°C for 2 hours. The reaction was
10 stopped by addition of 5 µL of 0.5 M EDTA and RNA was hydrolyzed by addition of 20
µl of 1 M NaOH at 70°C for 20 minutes. The reaction was neutralized with 25 µL of 1 M
HEPES and the cDNA was purified using QIAGEN PCR purification kits according to
manufacturer's instructions and eluted in nuclease-free water. The cDNA was concentrated
using Microcon30 spin columns (Millipore, Bedford, MA) and the volume retrieved dried
15 down under vacuum. The cDNA pellet was resuspended in 0.09 M sodium bicarbonate and
coupled to Cy3 or Cy5 monofunctional NHS ester reactive dye (Amersham). The coupling
reaction was conducted in the dark for 1 hour.

Dye-coupled cDNA was purified using Qiagen PCR purification columns, combined and
20 added to 10 µg of Human Cot1 DNA (Invitrogen Life Technologies). The cDNAs were
again concentrated with Microcon 30 spin columns (Millipore). The cDNA was hybridized
in a 40 µL volume containing the labeled cDNA, 20X SSC, 8 µg PolydA, 2.5x Denhardt's
solution, 4 µg yeast tRNA and 10% w/v SDS. The cDNA was then denatured at 98°C for 2
min and maintained at 60°C until required. 38 µL of the hybridization solution was applied
25 to a cover slip and then mounted onto an array slide. Hybridization was conducted in a
humid hybridisation chamber, in a hybridization oven, at 60°C for 16 hours. Following
hybridization the array slides were removed from their chamber and washed for 2 min in
each of a 0.5X SSC and-0.1% w/v SDS, 0.5x SSC and 0.01% w/v SDS, 0.6x SSC and
0.06% w/v SDS solution. The array slides were dried in a centrifuge for 1 min at 500xg.

EXAMPLE 56*Image Acquisition and Data Analysis*

Fluorescent images of the microarrays were acquired using a ScanArray Lite confocal laser scanner (Perkin Elmer) or GenePix 4000B scanner (Axon Instruments) and the images were analysed using GenePix Pro 4.0 and Acuity 2.0 (Axon Instruments) and GeneSight 3.0 (BioDiscovery, Sunnyvale, CA). Slides were scanned for both Cy3 and Cy5 signal at a 10 µM pixel resolution. Laser intensity and amplification of the photomultiplier tubes were adjusted to ensure approximately equal overall signal intensity for both Cy3 and Cy5. Data obtained from the scanner was imported into Gene Pix Pro (Version 4.0, Axon Instruments). False colour images were generated for each dye and combined to provide a representation of the relative Cy3 and Cy5 intensities. Individual cDNA spots were flagged if spot size was too small, if the overall signal intensity was too low, or if the Cy3 and Cy5 signal intensities within the spots were not linearly related. GenePix allows for the "flagging" of bad elements (defined by present GenePix parameters as feature signal intensity; feature background; element morphology; elements size and the percentage of pixels greater than feature background) that were then excluded from further analysis.

Median Cy3 and Cy5 signal intensities for each cDNA spot were imported from Genepix and data transformation conducted using Genesight (Version 3.0, BioDiscovery Inc, Los Angeles, USA). Signal intensities were corrected for local background and low expression values were omitted. The ratio of Cy3 to Cy5 was calculated and the data logarithmically transformed (base2). Signal intensity was normalized to the mean intensity of all respective signal intensities, providing a relative measure of gene expression for each element on the microarray slide. Gene expression analysis between control animals and animals separated for 4 days was assessed using an independent samples *t*-test. Differential gene expression as measured by microarray was screened for significance at *p*<0.05.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also

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includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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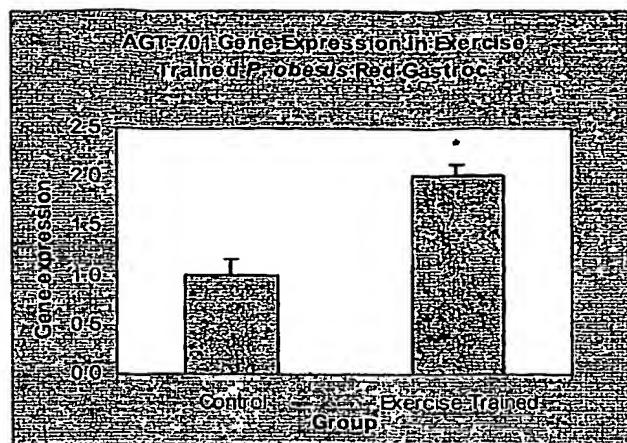
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ABSTRACT

The present invention relates generally to a nucleic acid molecule which is expressed in at least red gastrocnemius muscle of *Psammomys obesus* under particular physiological conditions. It is proposed that the nucleic acid molecule is differentially expressed under differing conditions of obesity, anorexia, weight maintenance, diabetes and/or metabolic energy levels. More particularly, the present invention uses microarray technology to identify genes which are expressed under particular physiological conditions. It is proposed that the subject nucleic acid molecule and/or its expression product be used in therapeutic and diagnostic protocols for conditions such as obesity, anorexia, weight maintenance, diabetes and/or energy imbalance. The subject nucleic acid molecule and its expression product or derivatives, homologs, analogs and mimetics thereof are proposed to be useful, therefore, as therapeutic and diagnostic agents for obesity, anorexia, weight maintenance, diabetes and/or energy imbalance or as targets for the design and/or identification of modulators of their activity and/or function.



*p<0.001

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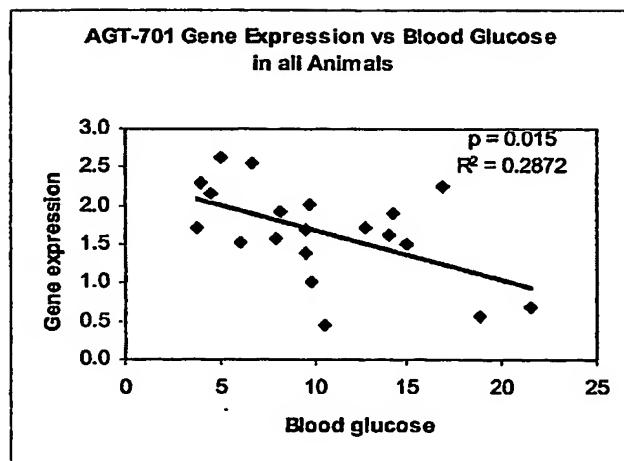


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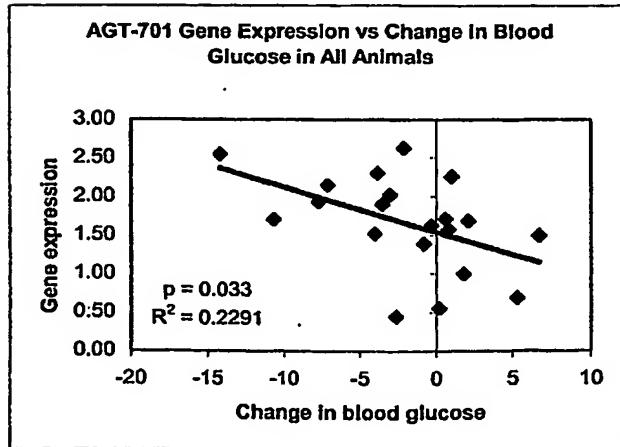
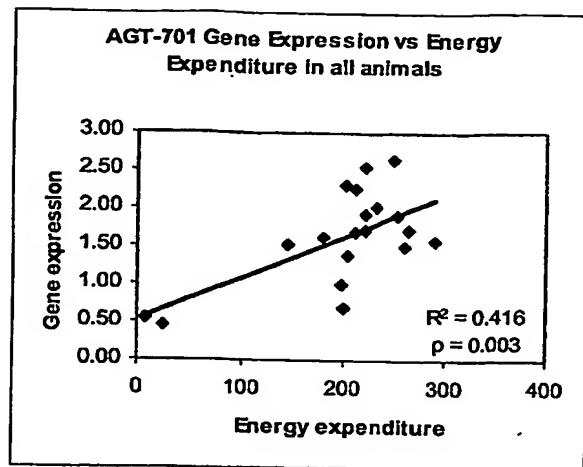
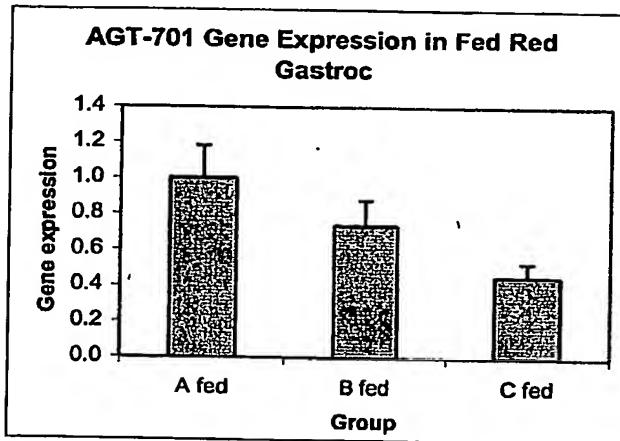
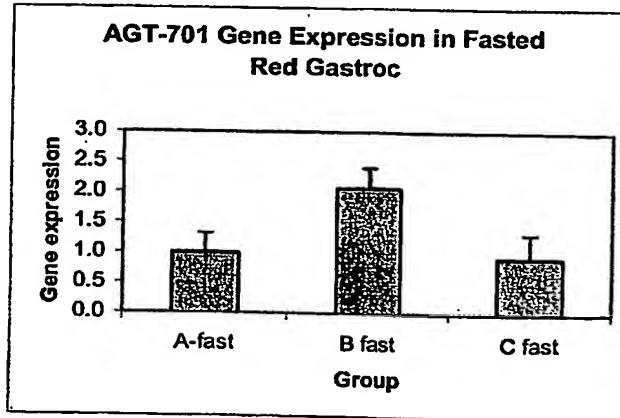
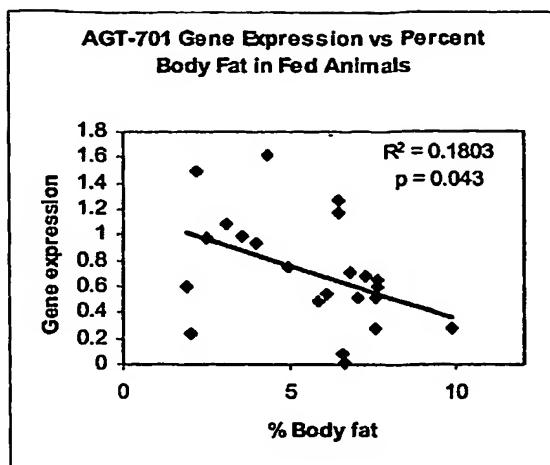
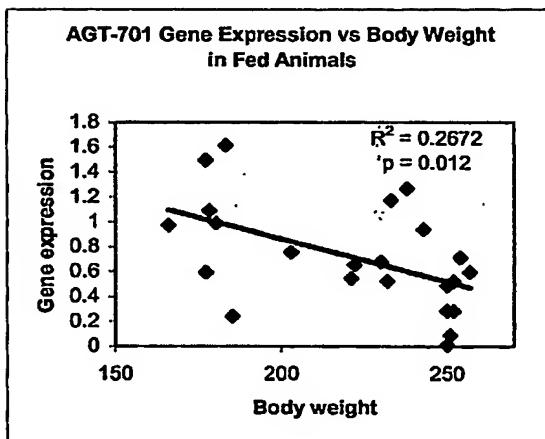
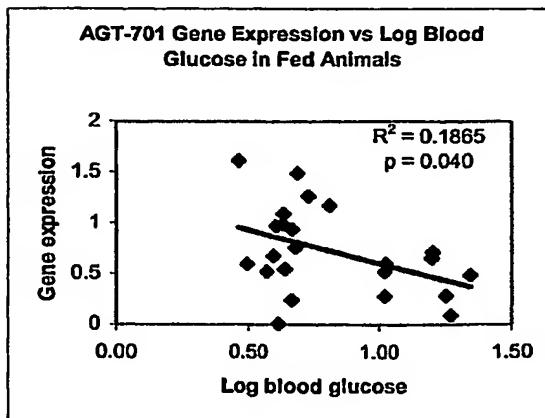
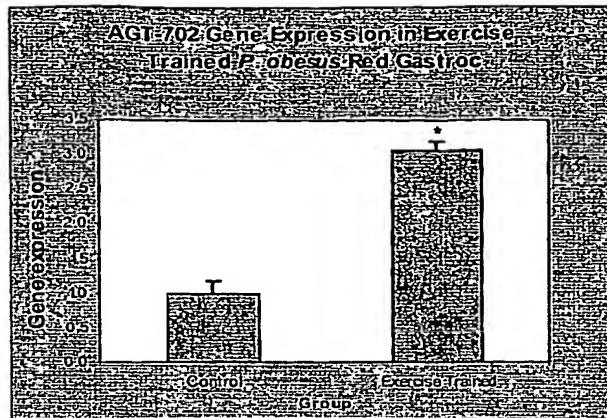


Figure 3

**Figure 4*** $p=0.011$ **Figure 5*** $p=0.04$ **Figure 6**

**Figure 7****Figure 8****Figure 9**



*p<0.001

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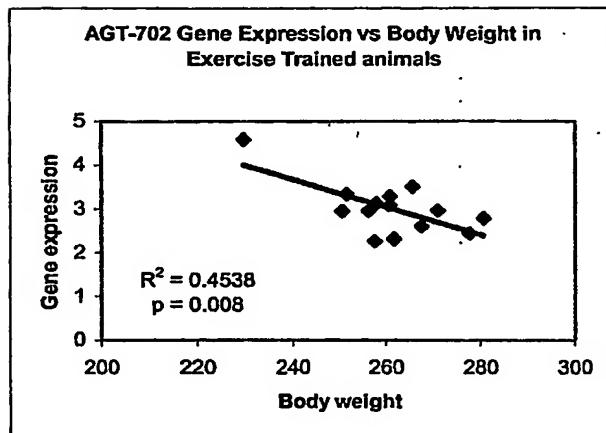


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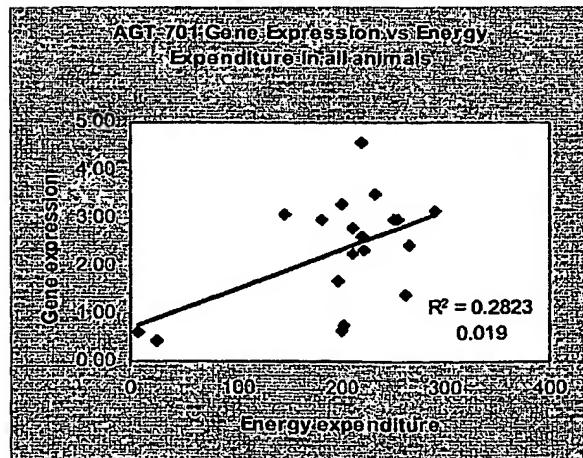
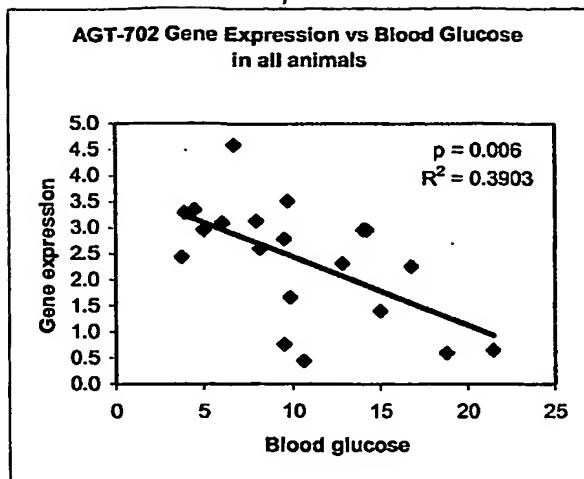
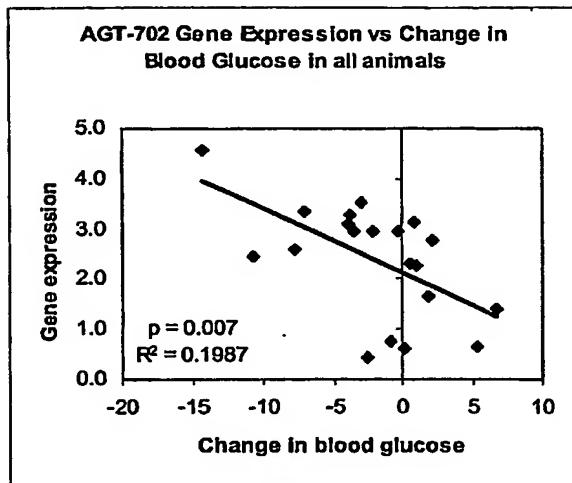
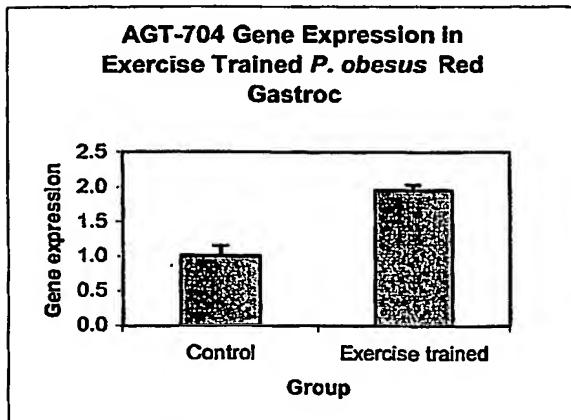
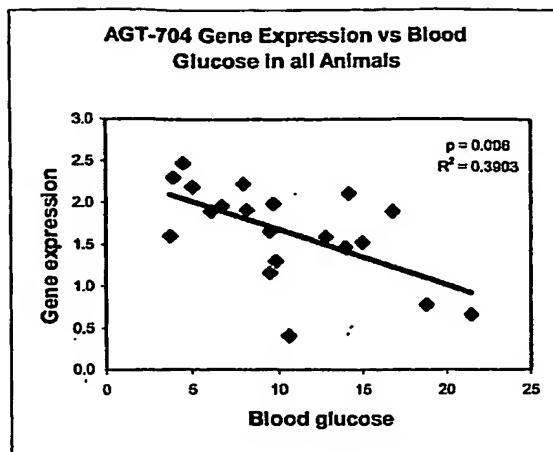
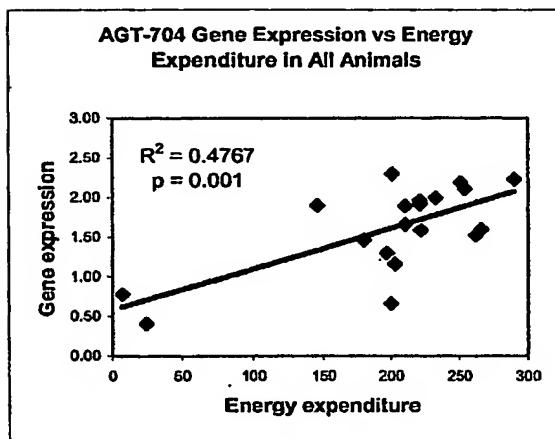
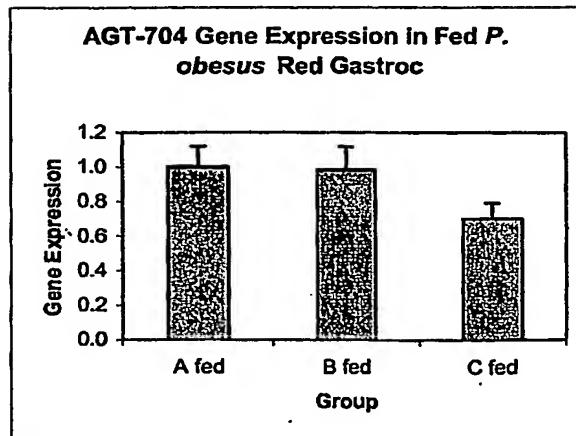
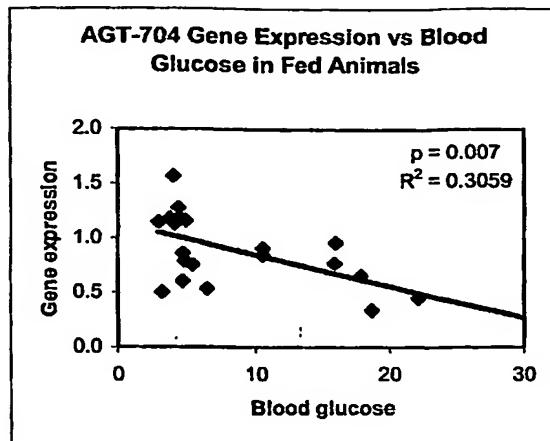
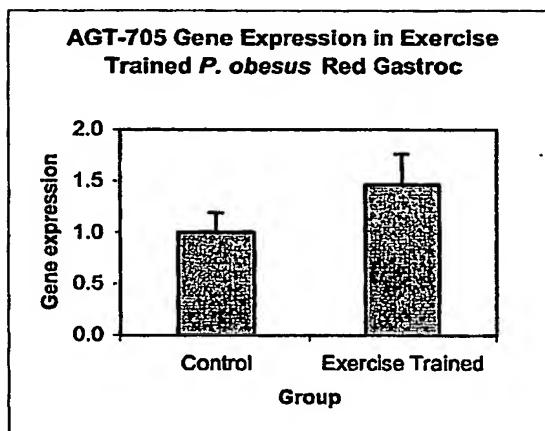
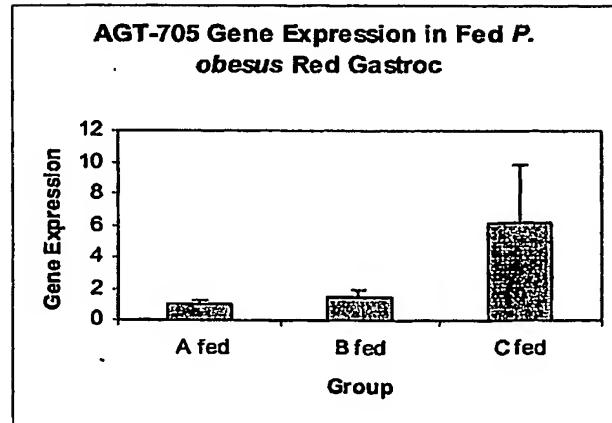
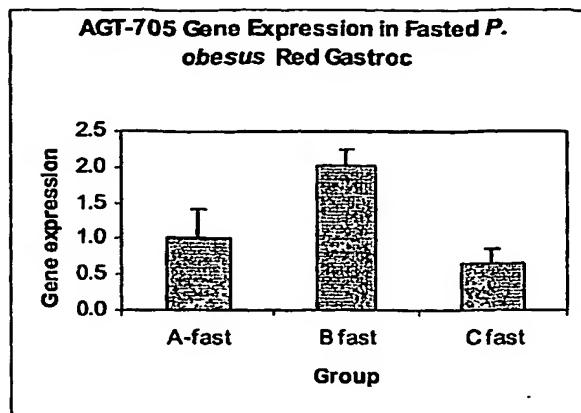


Figure 12

**Figure 13****Figure 14** $*p < 0.001$ **Figure 15**

**Figure 16****Figure 17****Figure 18**

**Figure 19****Figure 20****Figure 21**



*p=0.035, #p=0.007

Figure 22

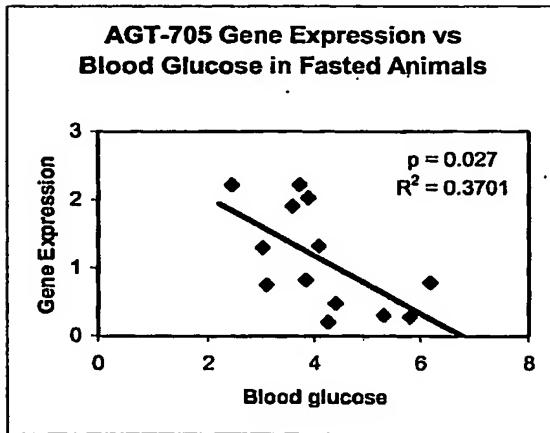
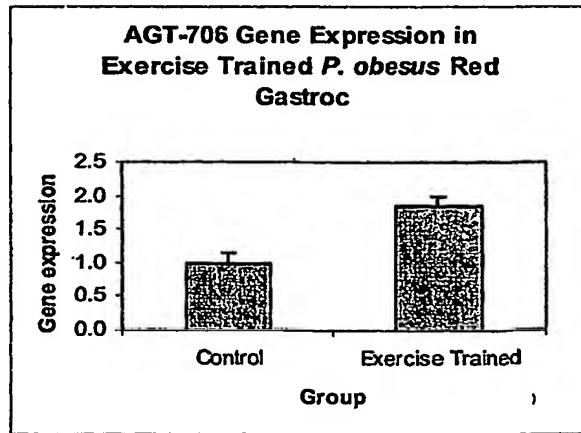
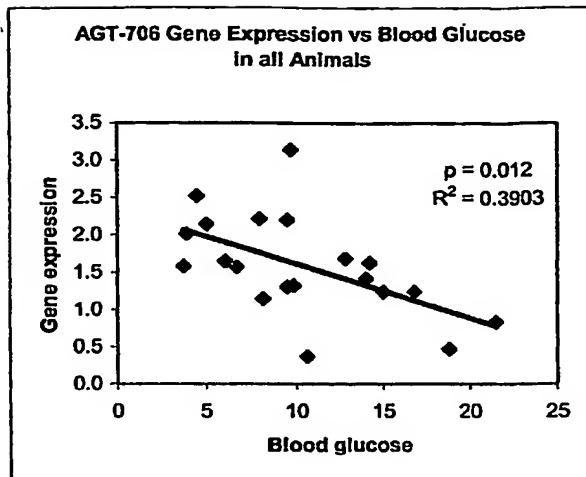
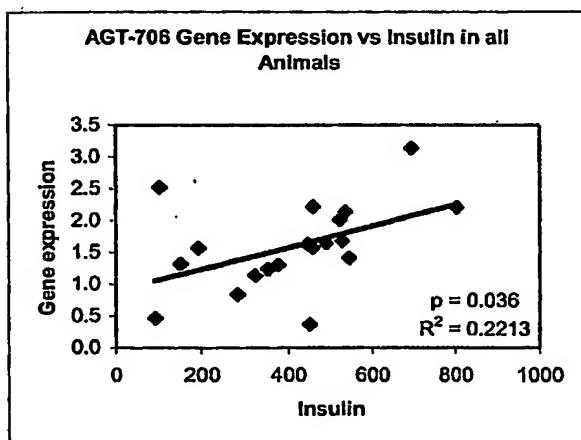
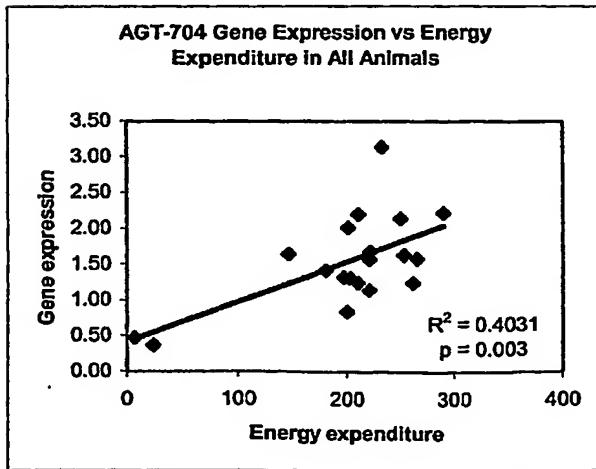


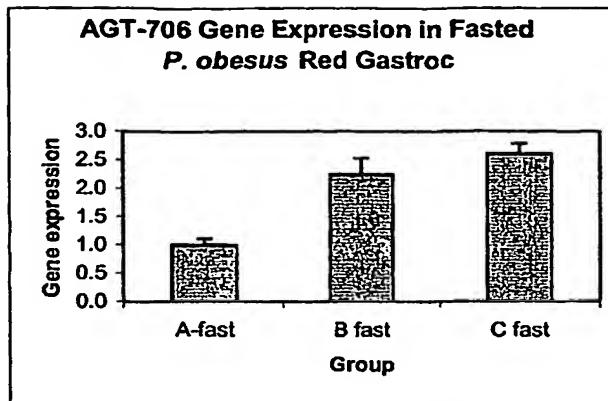
Figure 23



*p=0.001

Figure 24

**Figure 25****Figure 26****Figure 27**



*p<0.001, #p<0.001

Figure 28

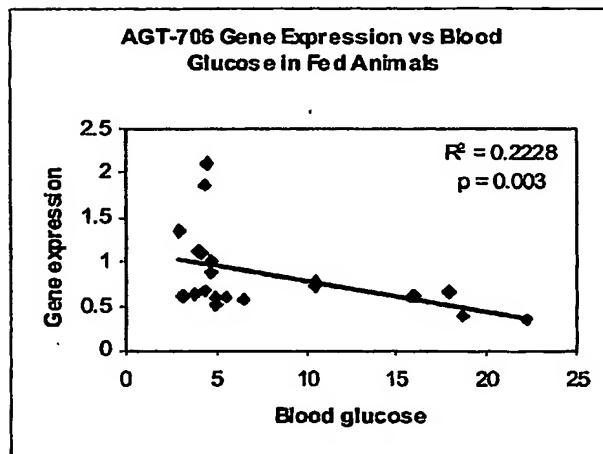


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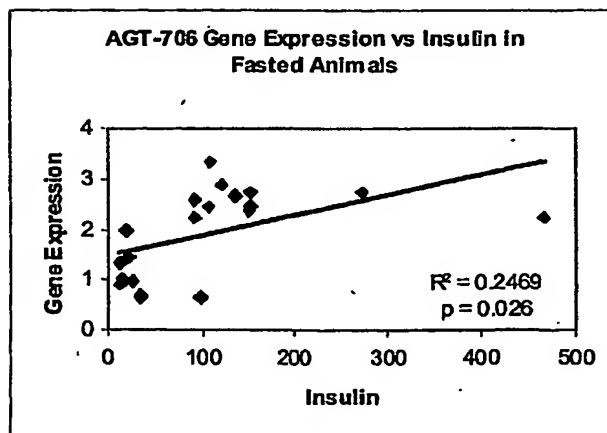
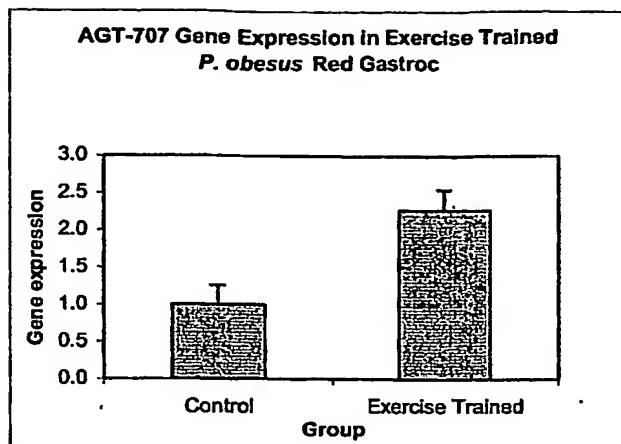


Figure 30



*p=0.037

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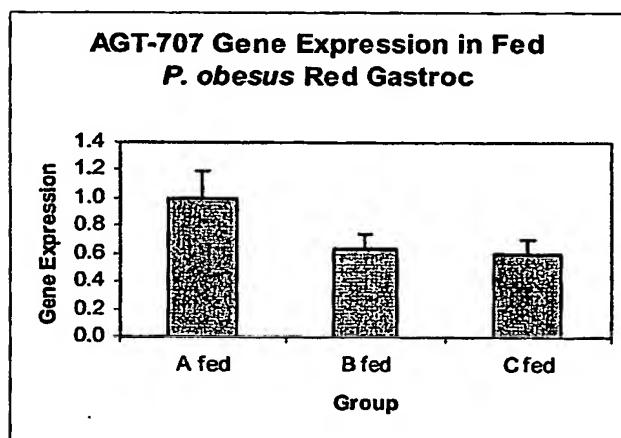


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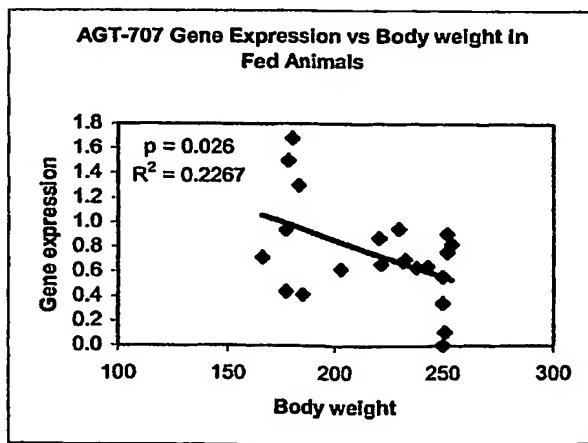
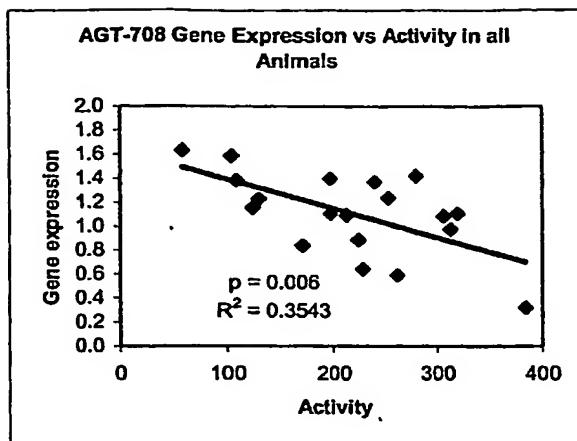
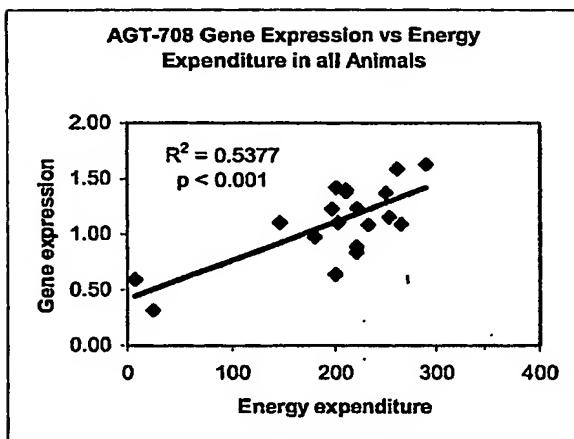
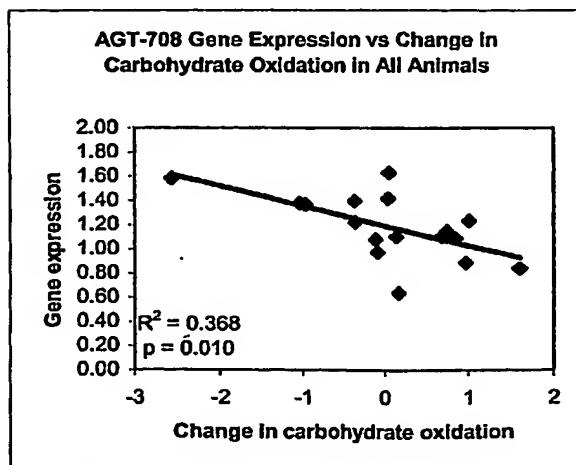
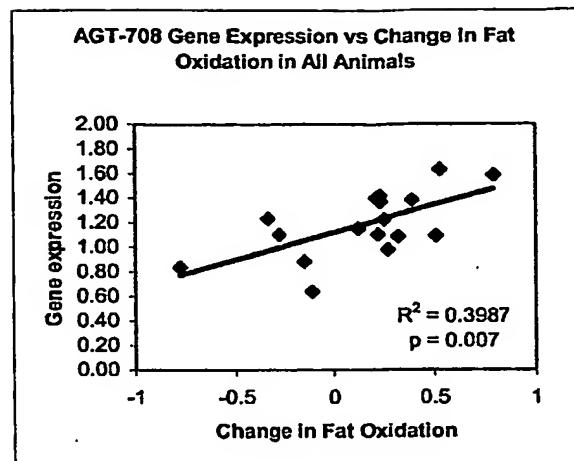
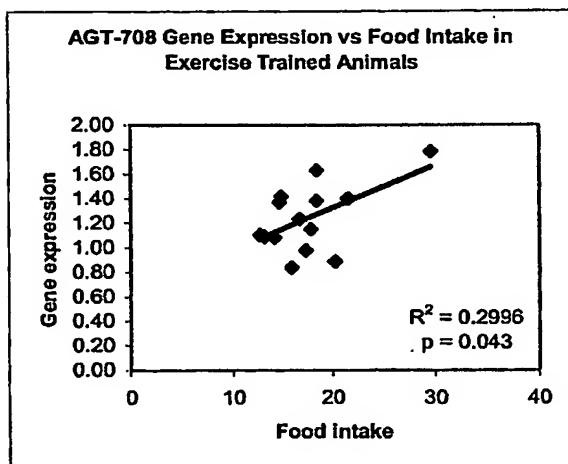
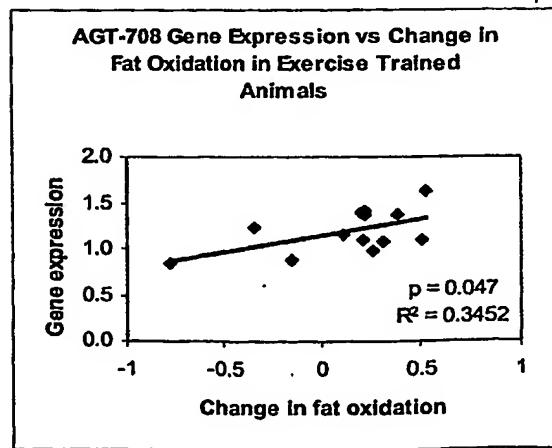
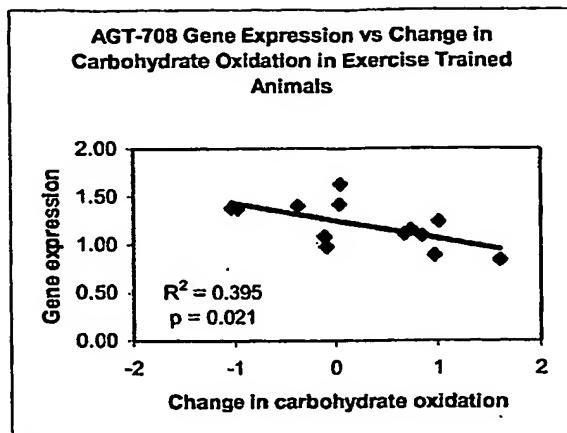
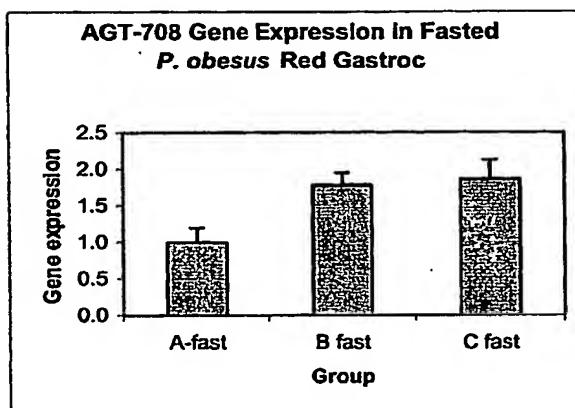
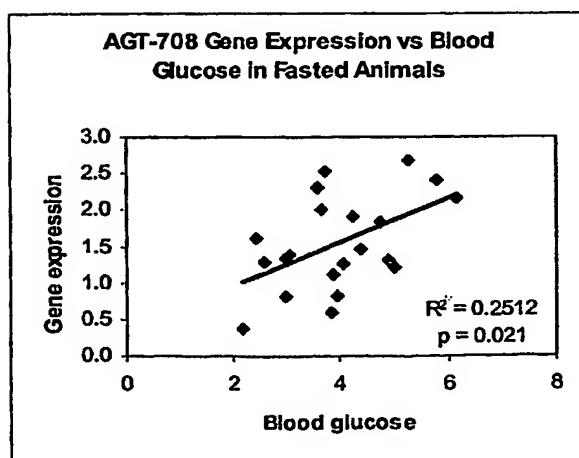
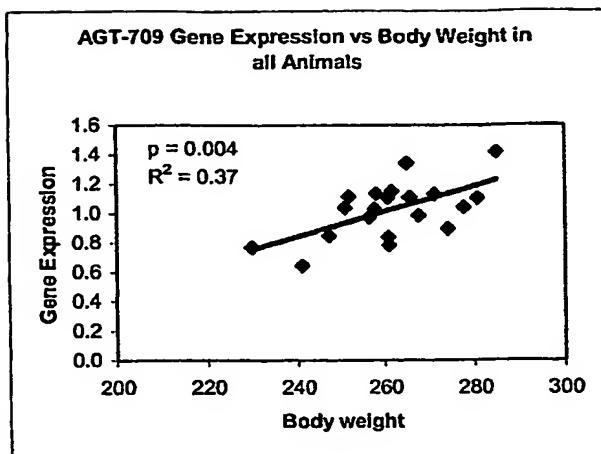
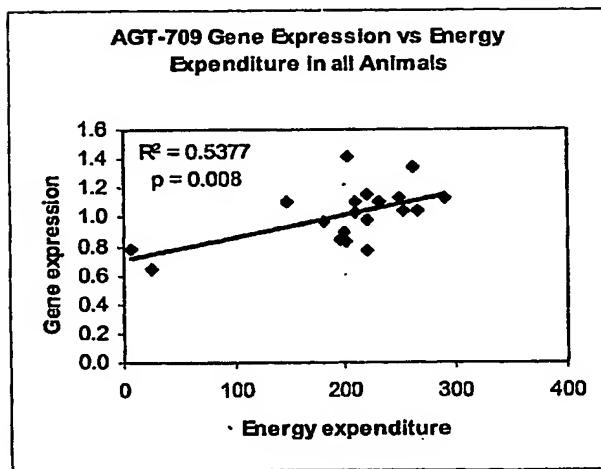
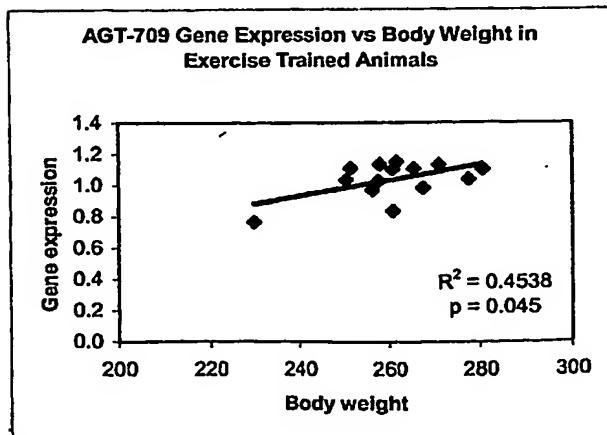


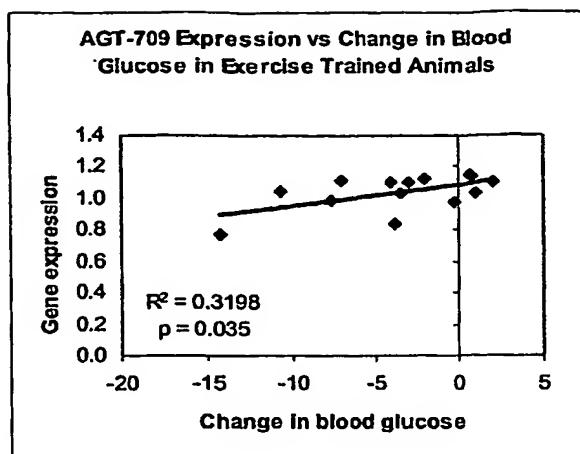
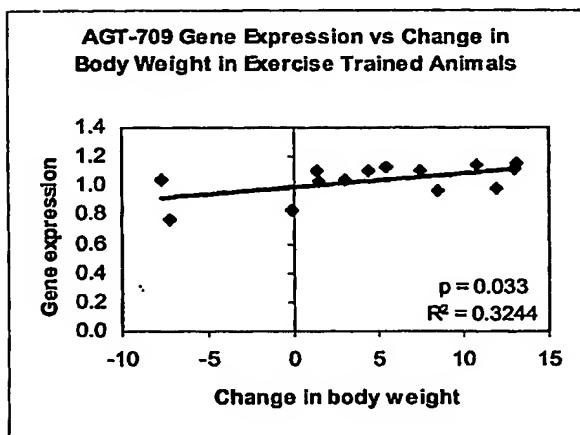
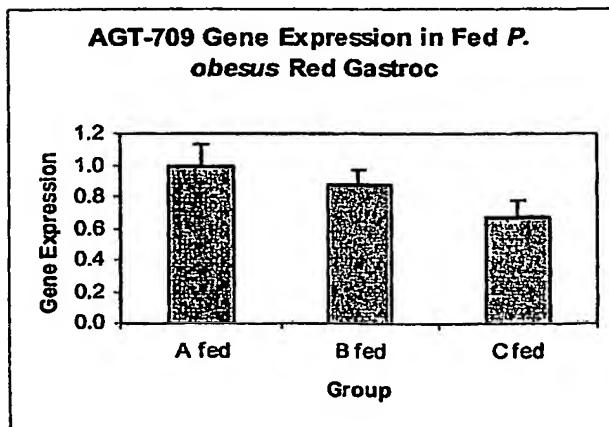
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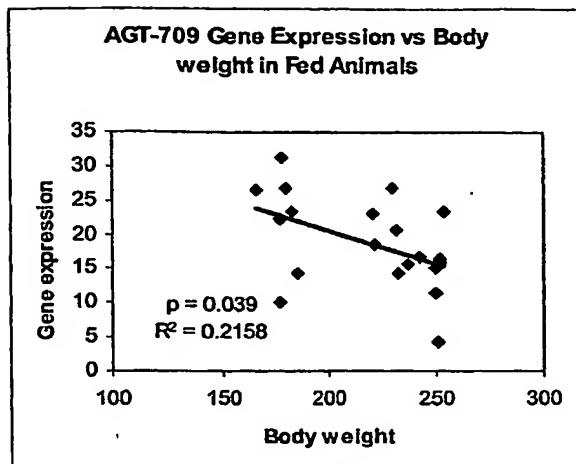
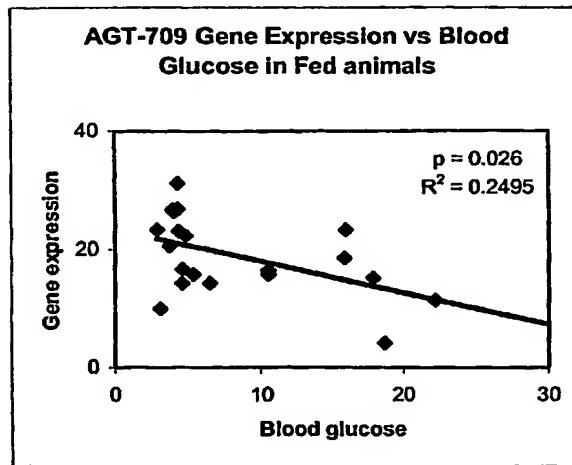
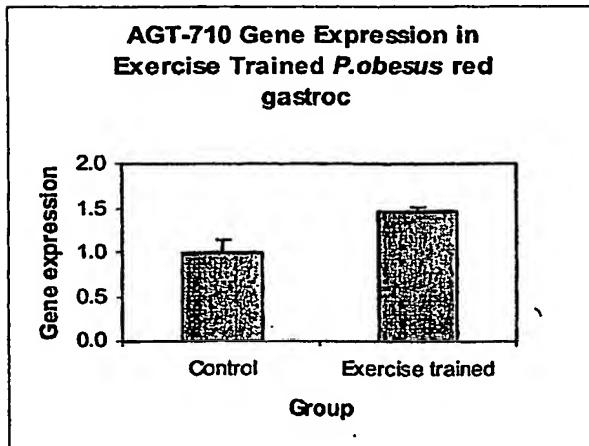
**Figure 34****Figure 35****Figure 36**

**Figure 37****Figure 38****Figure 39**

**Figure 40*** $p=0.01$ **Figure 41****Figure 42**

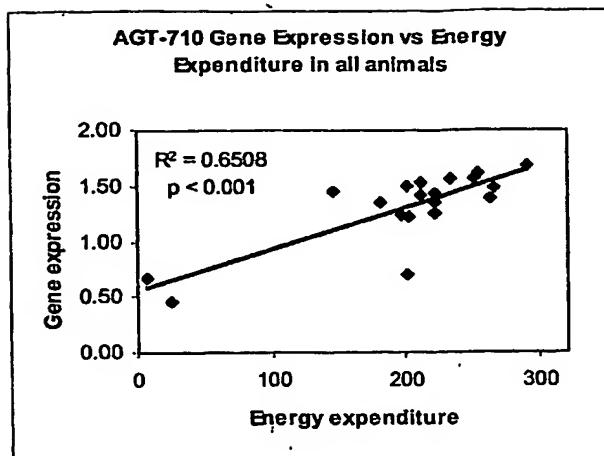
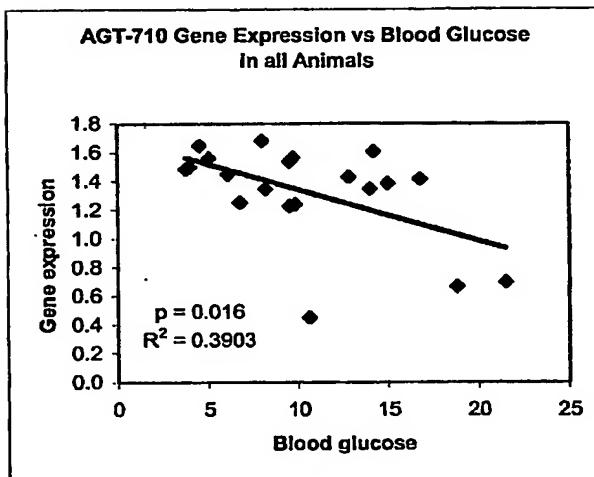
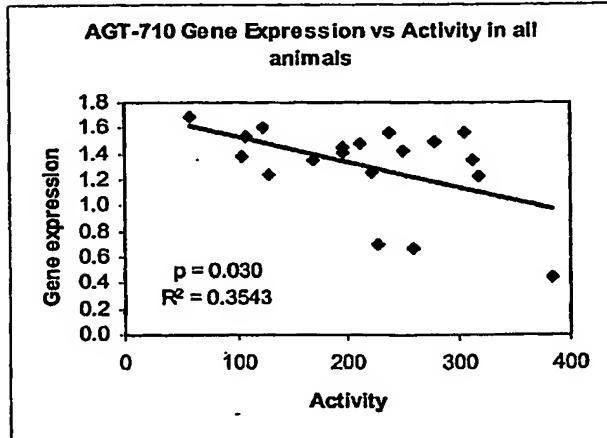
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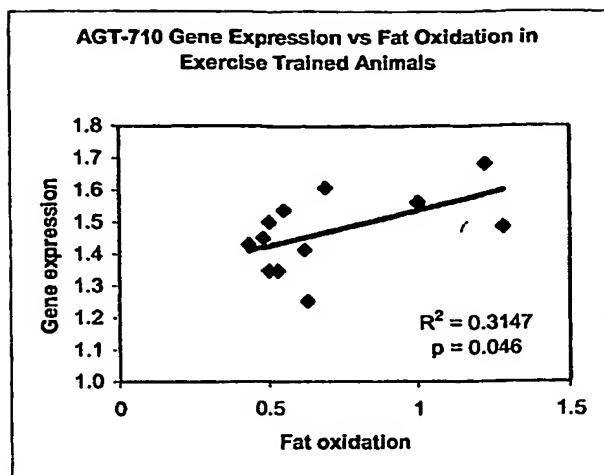
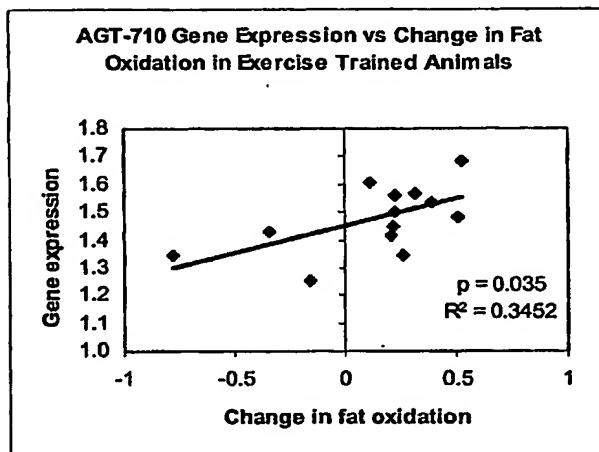
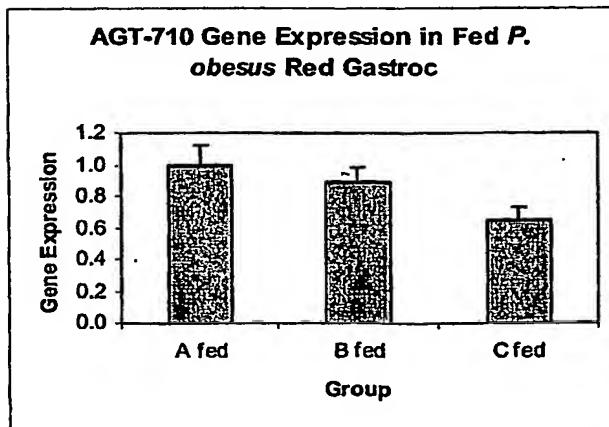
**Figure 46****Figure 47****Figure 48**

**Figure 49****Figure 50**

* $p=0.016$

Figure 51

**Figure 52****Figure 53****Figure 54**

**Figure 55****Figure 56**

* $p=0.021$

Figure 57

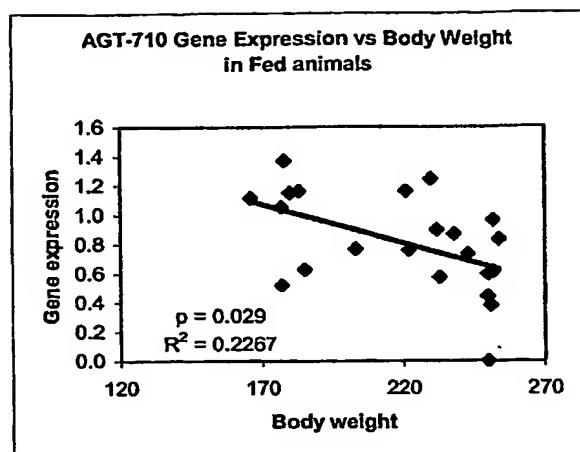


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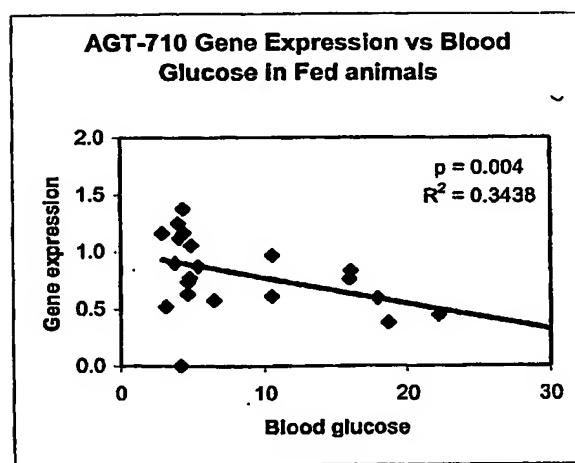


Figure 59

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acagcctgtg tctagtaaaa tagtttgc ggtgtgggtt ctatcttcc ataaaaaaagt	300
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- 4 -

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1020
1080

- 7 -

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aagaccacct gcagctgcag cctggcggtc cagctgtcta agggacgtga gagtgttcta      180
atcatttcca cagacccagc tcacaacatc tcagatgcat ttgaccagaa gttctccaag      240
gtgcctacca aggtcaaagg ctatgacaac ctcttgctta tggagataga cccgagcctg      300
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aaataaggtt ccggtaagat gcccagggtt catgagtatg gcacaaataa cagaggacag      240
gaggccttca cgacgaagga gcccgtaaat ggcctggagg gcacagatgc agttccaggt      300

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- 9 -

caagaaaaga gcagctttt caacaggcag tctgtggta tcatggAAC tcagcctgtc	360
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cctgttgata gactcaaAGC cgtgaacaga agcctttgg cctgtttcag acaatctctg	660
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